METABOLIC CHANGES FOLLOWING ADRENALECTOMY

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INTRODUCTION

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GENERAL

In recent years considerable interest has been aroused in the metabolic reaction of the body to trauma, particularly that due to surgery. As early as 1794 John Hunter in his Treatise on the Blood, Inflammation and Gunshot Wounds, clearly appreciated that the recovery from injury follows a recognised and ordered pattern. To quote from his work "There is a circumstance attending accidental injury which does not belong to disease - namely, that the injury done has in all cases a tendency to produce the disposition and the means of cure".

The knowledge since gained concerning the utilisation of fluid electrolytes, carbohydrates, nitrogen and fat in the immediate post-operative period has been of great value in enabling the surgeon to recognise or prevent metabolic abnormalities with greater precision than was previously possible. The results of the body's reaction to trauma are now well documented; of the manner in which these effects are produced very much less is known. Practically every body cell would appear to take part in the response but the fundamental biochemical changes in the cells have so far eluded identification, and the search for the primary stimulus to the resulting battery of changes still continues.

The literature on the various aspects of the metabolic response to surgical trauma is vast and formidable and covers many fields. This thesis is concerned with an attempt to shed

more light on the role played by the hormones of the adrenal cortex in this metabolic response and I have therefore selected from the literature those data which have seemed most relevant to this aspect. Likewise the body's response to the trauma of operation is compounded of numerous events, and only those which seem to be most closely linked with the hormones of the adrenal cortex, namely the alterations in nitrogen, sodium, and potassium metabolism, have been considered.

The operation of adrenalectomy presents perhaps the best available opportunity for studying the role of the adrenocortical hormones in the response to surgery. However, the number of patients presenting for adrenalectomy, in this hospital at least, is very limited. This means that intensive investigations have had to be carried out on a small number of subjects, but it is to be hoped that the results reported in this thesis will be corroborated by workers elsewhere.

2. NITROGEN METABOLISM AFTER TRAUMA

As early as 1872, Bauer observed that an increased elimination of nitrogen followed upon haemorrhage and Hawk and Giles in 1904 showed that the actual operation of venesection without the withdrawal of blood was enough to cause an increased urinary output of nitrogen. The increase was found to be in the amount of urea nitrogen excreted, thus suggesting that the source of the nitrogen was actual body protein, and did not merely arise from a failure to absorb dietary protein or amino acids. This conclusion was supported by subsequent findings of concomitant

increases in urinary sulphur, phosphorus and potassium excretion.

Increased nitrogen loss was also noticed by Benedict (1915) during prolonged starvation. Apart from the work of Wertheimer et al. (1919) who investigated the urinary nitrogen output during the first 24 hours in war injury cases and found a high excretion which was maintained for several days, interest in this increased nitrogen excretion flagged until Cuthbertson became interested in the subject in the 1930's.

His first paper in 1930 dealt with the increase in nitrogen excretion found in post-operative and post-fracture cases, this nitrogen loss reaching a maximum sometime between the second and sixth post-operative, or post-fracture, day and declining thereafter, the patients being allowed unlimited quantities of a restricted variety of foodstuffs. His studies on several immobilised patients convinced him that simple disuse contributed very little to the increased nitrogen excretion. However, he noted the similarity to the negative nitrogen balances found by Benedict (1915) and others in starved subjects.

In further studies, published in 1932, Cuthbertson established that anaesthesia alone did not produce a measurable nitrogen loss and that there was some correlation, but not as much as expected, between the extent of injury to the patient and the magnitude of the nitrogen loss. He concluded that the nitrogen loss was too great to come from the damaged cells alone and must be the result of generalised rapid protein catabolism to meet the body's immediate needs for tissue maintenance and repair. With a view to aiding the body in its work of tissue

repair he then tried, in 1936, the effects of supplying the patient post-operatively with a high nitrogen intake by means of intravenous administration of amino acids or high protein diets. He found that by these means he could modify the urinary nitrogen loss but could never prevent it entirely.

Albright, in his Harvey Lecture in 1943, introduced the concept of two partially antagonistic hormones produced by the adrenal cortex, namely the "S" or sugar hormone and the "N" or nitrogen-sparing hormone, the former being anti-anabolic, the latter anabolic. He postulated that the increased nitrogen excretion after stress of any kind, including operation, was due to an imbalance in the two hormones, there being a relative lack of the "N" hormone and excess of the "S" hormone.

The next step forward was made by Howard et al. who, (1944a), showed that the increased urinary nitrogen excretion was much greater and sustained much longer after fractures than after operations on the femur thus showing that the extent of tissue damage was of importance. They also verified Cuthbertson's findings that anaesthesia, bed-rest, disuse atrophy, fever and infection made only minor contributions to the increased nitrogen loss. In a continuation of this work, Howard et al. (1944b) came to the conclusion that nitrogen excretion after operation showed great variation from patient to patient whereas after fractures the nitrogen excretion was relatively constant from patient to patient, suggesting that some unknown but relatively specific factor was of importance in determining the extent of the negative nitrogen balance found in the post-operative period. That

this factor might be a principal of the adrenal or pituitary glands was first suggested by Albright and Browne (1943). Of two of Howard's patients of almost identical height and weight, and age, and undergoing the same operation, one was force-fed nitrogen in the form of amino acids in the post-operative period, while the other was given no supplementary nitrogen resulting in a difference in intake of 50g. of nitrogen and 8000 calories over the first ten post-operative days - and yet the net nitrogen losses of these two patients were found to be practically identical, showing that the excess administered nitrogen had been excreted quantitatively. These workers also made the interesting observation that one patient who was cachetic from long-standing rheumatoid arthritis failed to show an increase in nitrogen excretion following operation, thus confirming the results of Cuthbertson's experiments on injury to protein-depleted rats. Subsequently many workers substantiated this finding that previously depleted patients showed little or no increase in nitrogen excretion after operation, their results being reviewed by Peters in 1944. Peters concluded on the available evidence, that the healthy patient, as opposed to the malnourished individual, invariably suffered post-operative protein depletion which could not be prevented by any known dietary measures and that this depletion was due to the fact that protein synthesis was in abeyance.

Notwithstanding the conclusions of this review Werner (1948) maintained that in the immediate post-operative period the nutritional intake was invariably inadequate and that nitrogen

balance could be achieved by supplying the requisite calories and proteins.

Thus at this stage there were three theories current as to the reason for the increased amounts of nitrogen excreted after operation - Cuthbertson and Howard thought that the nitrogen came from increased catabolism of tissue protein, Peters and Albright considered that the nitrogen was excreted because protein synthesis was in abeyance, and Werner maintained that it arose from the fact that insufficient amounts of protein and calories were being supplied to the body. The theory that adrenal steroids were anti-anabolic gained confirmation from the work of Clark (1950) who was able, by means of glycine labelled with isotopic nitrogen, to demonstrate decreased protein synthesis in rats given cortisone. On the other hand Hoberman, in the same year reported both a stimulation of protein catabolism and an inhibition of anabolism in fasting animals given adrenal steroids, again using labelled glycine.

Meanwhile Ingle had been investigating the role of the adrenal gland in the production of the negative nitrogen balance after injury. That the adrenal cortex played a part in protein metabolism was first shown by Long et al. in 1940. They administered adrenocortical extract to fasted normal, adrenalectomized, and hypophysectomized rats and found in all cases an increase in nitrogen excretion. Ingle in 1947a showed that normal and previously adrenalectomized rats maintained on adrenocortical extract, showed a marked increase in urinary nitrogen following fracture of one hind leg. On the other hand, adrenalectomized

rats maintained only on saline showed not such rise in nitrogen in nitrogen excretion after fracture of a hind leg. He concluded that the negative nitrogen balance found after fractures required the presence of adrenocortical hormones but was not caused specifically by the increase in secretion known to occur following stress of any kind. This was an example of the so-called "Permissive theory" of the action of the adrenal hormones of which Ingle, although not the originator, has long been one of the principal exponents.

Ingle's rather unsatisfactory experimental results were confirmed in a rather more convincing manner later in 1947 by Toby and Noble who found that the clamping of a limb in a normal rat caused a marked increase in nitrogen excretion which was not increased further by the administration of adrenocortical extract. Large doses of adrenocortical extract without trauma caused only a slight increase in nitrogen excretion. On the other hand, adrenalectomized rats maintained on desoxycorticosterone acetate (DCA) and/or saline showed no increase in nitrogen excretion after limb-clamping whereas adrenalectomized rats maintained on adrenocortical extract showed a normal nitrogen response. Administration of the same dosage of adrenocortical extract to untraumatized adrenalectomized rats elicited no nitrogen response whatsoever. They therefore concluded with Ingle, that adrenocortical hormones were not, per se, responsible for, but their presence was necessary for, the increased protein catabolism which followed trauma.

The theory that the nitrogen excretion was due to starvation

alone was not held for long, despite the fact that Co Tui et al. (1944) claimed to have entirely abolished the phase of negative balance after gastrectomy by the intravenous administration of large quantities of both amino acid mixtures and protein hydrolysates, a claim supported later by the work of Abbott and co-workers, summarised by Holden et al. (1957), who, by completely maintaining nitrogen and calorie intake by balanced intravenous intake of amino acids, fat emulsions, and dextrose, reduced the net loss of nitrogen after major operations to the relatively small amount of 9g. Wilkinson et al. (1950) were unable to confirm Co Tui's findings and, moreover, found in control subjects who were subjected to the same dietary restrictions as patients undergoing gastrectomy, that the level of urinary nitrogen fell rapidly after the restriction of food intake, in contrast to post-operative patients in whom urinary nitrogen levels actually rose. These authors suggested that the negative nitrogen balance of the catabolic phase was mainly due to some effect of the trauma on the metabolic processes and that the inevitable restriction in food intake played only a very minor part - increasing the intake of nitrogen by intravenous infusion of protein hydrolysates and oral protein-enriched milk only increased the gross amount of nitrogen excreted but did not alter the overall balance. These results were corroborated by Moore and Ball (1952) in their extensive balance studies. These authors reiterated the opinion that this nitrogen response to surgery was physiological and not pathological and should therefore not be interfered with unless abnormally prolonged.

Despite this, Flear and Clarke in 1955 tried to abolish the nitrogen response in their patients by transfusion of large amounts of whole blood but only succeeded in postponing and diminishing it.

Engel (1952) had come to the same conclusion after investigating the effects of acute stress on urea formation in nephrectomized and nephrectomized-adrenalectomized rats. In nephrectomized rats and nephrectomized-adrenalectomized rats maintained on adrenocortical extract he found an immediate increase in urea formation following acute stresses of various kinds. This response was completely lacking in nephrectomized-adrenalectomized rats maintained only on saline and DCA. Acute or chronic pretreatment of nephrectomized rats with massive doses of adrenocortical extract of ACTH had no effect on the magnitude of the increase in urea formation following stress. The conclusion seemed inescapable that the adrenal hormone was necessary but not responsible for the nitrogen response and Engel suggested that the hormone in some way sensitized the organism to respond to stress with a characteristic metabolic pattern.

In 1953, Cuthbertson working along the same lines endeavoured to find out if the protein catabolic effects of injury and cortisone were comparable and to discover what happened when the two were superimposed. He implanted one or two 25mg. tablets of cortisone acetate in the hind limbs of rats and compared the results with those obtained after fracture of the hind limbs. He found that one pellet or fracture of one hind limb had roughly

equivalent effects on nitrogen excretion and that two pellets or one pellet plus the fracture of one hind limb had very much greater effects which were not great enough to be additive but showed that increasing the stimulus had obviously increased the response. These findings could only be reconciled with Ingle's fracture experiments on adrenalectomized rats if it was presumed that the effect of injury was not to evolve a liberation of hormones from the adrenal cortex but to cause some change in the circulating hormone which then induced the catabolic phenomenon. This was the first mention of a concept which later proved to be of major importance.

The picture which remains is of a phase of negative nitrogen balance after operation, presumably resulting from a combination of decreased protein anabolism and increased catabolism, which is invariably present in well-nourished patients and which at its height can be modified but cannot be entirely abolished by any parenteral, oral or intravenous administration of any form of nitrogen-containing compound now known. It must, however, be borne in mind that the nitrogen response may be lacking in malnourished individuals who have no expendable body nitrogen stores. This raises the question as to whether this response is an intrinsic part of the metabolic response to trauma if it can thus be dispensed with when necessary. It must also be remembered that no patient undergoing an elective operation is leading a life which can be regarded as normal from the point of view of diet and exercise in the period immediately preceding operation. It is known that the presence of the hormones of the adrenal

cortex is essential for the nitrogen response to occur although they do not themselves initiate the response. However it is not known how much of the excreted nitrogen may be accounted for by the nitrogen of the red blood cells extravasated at the site of any but the most minor surgical procedure. The method of balance study, measuring as it does only net difference between input and output and not the actual sources of the substances, will never give the answer as to the source of the extra nitrogen excreted after trauma. And until we know the source of this nitrogen we cannot say for certain whether or not a change in nitrogen metabolism is an intrinsic part of the response to surgery. The question remains open.

3. POTASSIUM METABOLISM AFTER TRAUMA

Benedict, in his classical starvation experiments in 1915, noted that the urinary nitrogen loss was accompanied by a loss of potassium, the ratio of the two elements being reasonably constant and of the order of 10:1, corresponding fairly closely to the potassium:nitrogen ratio of "wet lean tissue" and therefore suggesting as its source the breakdown of whole tissue for energy purposes.

Due perhaps to the tediousness of the methods for potassium determination before the advent of the flame photometer, research into urinary potassium excretion flagged until the 1940's although Cuthbertson in 1930 had noted an increase in potassium output post-operatively. For some time it was assumed that this potassium was merely a necessary adjunct to the increased nitrogen excretion and had no intrinsic significance (Howard, 1943; Reifenstein, 1944; Limbert et al., 1945). However, Stewart and Rourke (1942) and Howard himself in 1946, pointed out the fact that immediately following operation the output of potassium was much greater than expected from the nitrogen output. That urinary potassium excretion was increased in the first 24 hours after operation was again clearly shown in 1948 by Berry et al., a finding confirmed by Blixenkrone-Møller (1949) who concluded that the excretion rate bore a rough relationship to the magnitude of the operation, no change in potassium excretion at all being observed in two patients undergoing simple hernia repairs. The latter also showed that standard pre-operative diet and medication had no effect on the potassium excretion of normal persons

whereas venesection caused an increase. Blixenkrone-Møller noted too that the increase in potassium excretion in the first 24 hours after operation was greater than that expected from tissue breakdown alone, a fact that had been observed in mice subject to burns and tourniquet trauma by Rosenthal and Tabor (1945) who found that potassium excretion in the first two post-shock days was well above that of the control uninjured mice. Determination of the potassium content of the uninjured hind legs after trauma by these authors showed a decrease of almost one third as compared with the uninjured control hind legs, a decrease which nonetheless only accounted for about one half of the total increase in potassium excreted, the rest of which was concluded to have come from the non-traumatized areas. These authors also noticed a gain in sodium content of the injured leg roughly equivalent to the amount of potassium lost, suggesting an exchange of cell potassium for sodium - a possibility first demonstrated in vivo by Manery and Solandt (1943) whose work arose from the much earlier finding of Schmidtmann and Mathers (1927) that there was an increased cell membrane permeability in the cells surrounding damaged tissue which led to loss of potassium from the cytoplasm.

Conflicting results were obtained in human patients after operation by Winfield et al. (1951) who took muscle biopsies at the site of operation immediately before and after the operation in a series of patients undergoing various abdominal operations, control biopsies being taken from neighbouring abdominal muscles. They found that the muscle at the site of operation lost approximately 55% of its potassium whereas the neighbouring muscle

lost very little potassium except in the case of one very prolonged operation. They concluded that the muscle potassium loss tended to parallel the extent of trauma, undamaged muscle contributing only minor amounts to the increased potassium excretion. It may be, however, that their muscle biopsies were taken too soon after operation for the potassium changes to have attained their maximum and that the prolonged operation alone presented the true picture. This might explain the discrepancies between their results and those obtained by Schilling et al. (1953) in dogs from whom similar muscle biopsies were taken from healing abdominal wounds. These latter authors went on to observe the effects of ACTH and found that the potassium losses were markedly exaggerated both in the wounded areas and in the normal control muscle. They therefore came to the conclusion that, unless the area of injury was large, most of the observed increase in urinary potassium came from normal tissue under the influence of increased adrenocortical activity.

The parallel between increased adrenocortical activity and loss of potassium from the body was first noticed by Selye, both being components of his "alarm reaction" (1940) which will be referred to again later. Albright, too, in his Harvey Lecture of 1942 commented on the low serum potassium values sometimes found in Cushing's syndrome which was known to be associated with adrenocortical hyperactivity of some description. However, it was some time before it was realised that the body stores of potassium could be greatly depleted without the serum potassium being reduced and this, as well as the time taken for potassium

estimation by the classical chemical methods, tended to deflect interest from the role played by potassium in the body, a role now known to be of primary importance.

Again, as in the case of nitrogen, it was Ingle (1951a) who threw the first light onto the relationship of the adrenal cortex to the increased potassium excretion after trauma. He subjected normal rats and adrenalectomized rats maintained on constant doses of adrenocortical extract, to fracture of both hind legs and in both groups demonstrated a post-injury rise in the excretion of potassium showing that, although the presence of adrenocortical hormones was required for the increase after injury, no increase in their secretion was necessary, i.e. the adrenal cortex once again played a supporting and not an initiating role. These experiments of Ingle's have recently been repeated by Share and Stadler (1958) who also found that only approximately one half of the potassium excreted could be accounted for by the potassium lost from the injured tissues. As increased adrenal hormone secretion could not be incriminated they postulated a "toxic factor" liberated from the site of the tissue damage which acted on the uninjured cells causing them to release some of their potassium, a conception put forward earlier by Wilkinson et al. (1951).

Finally, Flear and Clarke (1955) in their studies on patients transfused at operation with large amounts of whole blood, claimed to have greatly reduced the potassium loss in the first 24 hours following operation, although the impression gained is that they had obliterated rather than reduced the loss.

And there, at the present, the knowledge of post-operative potassium metabolism remains. That there is normally a marked negative potassium balance in the two days immediately following operation is not in doubt, although Flear and Clarke have shown that it may be reduced. What is in doubt is the origin of this potassium. Does it come merely from the breakdown of damaged tissue and the lysed red blood cells at the site of injury? The fact that the urinary potassium:nitrogen ratio is much higher post-operatively than the ratio in "wet lean tissue", and that the negative nitrogen in most cases persists so much longer than the negative potassium balance would appear to be against this. On the other hand, these discrepancies may simply be accountable for on the basis of the known difference in the rates of movement of these two elements out of and into individual cells. Or does the potassium excreted post-operatively come partly from the uninjured tissue cells of the rest of the body under the influence of some factor - the "toxic factor" of Share and Stadler - since Ingle has so clearly shown that it still occurs when there can be no increase in the secretion of adrenocortical hormones?

As in the case of nitrogen we are left with the as yet unanswered question - Is this increased potassium excretion after operation an integral part of the metabolic response or is it a more or less accidental by-product?

4. SODIUM METABOLISM AFTER TRAUMA

That oliguria frequently follows surgical operations has been known since the beginning of this century (Pringle et al., 1905). The very low urinary output of sodium which was also known to follow operation was long thought to be inextricably linked with the oliguria and indeed both were thought to be mere consequences of the dehydration which of necessity precedes all elective major surgical procedures, together with the decrease in glomerular filtration known to occur.

The lack of realisation that this post-operative conservation of water and sodium by the body was purposeful, led most workers to administer large volumes of sodium chloride of varying tonicities to patients during and immediately following operations, despite the warnings of Evans (1911) and Trout (1913) who pointed out that these patients would not tolerate large volumes of infused saline and often developed massive oedema, sometimes with fatal results. In 1938 Coller et al. enunciated their infamous "clinical rule" which gave a rough guide to the amount of saline to be infused into the surgical patient, recommending an additional arbitrary litre of isotonic saline on the day of operation over and above volume for volume replacement. This "clinical rule" was unhappily drawn up on the assumption that the amount of chloride excreted in the urine gave an exact measure of the amount of sodium excreted - urinary chloride being much more easily measured than urinary sodium in the days before the advent of the flame photometer. This assumption has since been proved to be

completely erroneous, Goldzieher and Stone (1949) and Denton et al. (1951) showing that chloride and sodium were regulated quite independently of each other. Also Coller himself, in 1940, showed that the urine chloride content did not necessarily reflect the body content of sodium and chloride.

However saline continued to be administered practically routinely to all surgical patients until 1944 when Coller et al. published a forcible statement of the evil effects of the administration of excess saline to the post-operative patient, following this in 1945 by a demonstration that, on the average, the post-operative patient retained 53% of the sodium and 19% of the water given as intravenous isotonic saline, resulting in marked intracellular dehydration, fluid being removed from the cells in order to maintain extracellular isotonicity. This led to the suggestion that hypotonic saline be used in an effort to redress the balance between the amounts of sodium and water retained - a suggestion which, fortunately, did not gain much popularity.

With the realisation that the sudden post-operative fall in urinary output of sodium occurred even in the face of administration of large amounts of sodium, came the realisation that this was not a simple consequence of decreased or even zero, sodium intake. The post-operative fall in urinary sodium output was shown to occur within the 24 hours immediately following the operation and to last for several days (Wilkinson et al., 1949; Moore and Ball, 1952; LeQuesne and Lewis, 1953) although LeQuesne and Lewis, as will be mentioned later, claimed to be able to divide the period of sodium retention into two distinct phases.

On the other hand, the effect on urinary sodium excretion of simple withdrawal of salt from the diet of a healthy person (Benedict, 1915; Moore and Ball, 1952; Howard et al., 1946; Renwick et al., 1955) was shown to require several days before its full effect could be felt and the urinary sodium levels fall to those normally encountered immediately following operation. Thus the conservation of sodium following operation was shown to be a consequence of the operation itself and not merely of the lowered sodium intake usually found pre-operatively. Also Coller et al. (1943) and Moyer (1950) showed by anaesthetizing healthy subjects, that the anaesthetic per se did not cause conservation of sodium and water by the kidney. It was clearly seen, as a consequence of this accumulation of facts, that there was no need to administer sodium chloride in this period unless to compensate for extra-renal losses, e.g. through prolonged vomiting, gastric suction, fistula, etc..

An interesting fact was brought to light by Limbert et al. (1945) and Cooper et al. (1949) who discovered that the rate of sodium excretion was greater in the oliguric phase than in the succeeding phase when a very dilute urine was elaborated, thus clearly differentiating the two phenomena. This differentiation was confirmed in 1951 by Holland and Stead who studied the effects of pitressin injections on the water, sodium and chloride excretion of healthy persons. They found that, while there was a marked oliguria due to increased tubular reabsorption of water with alteration in the glomerular filtration rate or renal plasma flow, the pitressin injection had no demonstrable effect on the

rate of excretion of sodium, potassium or chloride.

That there was a decrease in glomerular filtration rate and renal plasma flow following operation was suggested by Elman et al. (1949) on the grounds of the noted post-operative fall in creatinine output, and that this fall in glomerular filtration rate would reduce the excretion of sodium was shown by Chalmers et al. (1952). Ariel and Miller (1950) suggested that this reduction in glomerular filtration rate and renal blood flow was a result of the hormonal imbalance consequent on surgery. However, the phase of oliguria had repeatedly been demonstrated to be very transitory in nature, rarely lasting for more than 24 hours, whereas the period of sodium retention invariably lasted for several days. The post-operative changes in renal haemodynamics could therefore only play a very small part in the prolonged conservation of sodium and it appeared that the major role must be attributed to hormonal factors.

Ingle in 1951 showed that there was no difference in the amount of sodium retention after trauma in non-adrenalectomized rats and adrenalectomized rats maintained on adrenocortical extract, thus demonstrating once again that the metabolic response to trauma was not mediated by a change in the secretory activity of the adrenal cortex. On the other hand, he showed that little or no retention of sodium occurred after trauma in adrenalectomized rats maintained on saline alone, thus showing that adrenocortical hormone was necessary for the response to occur.

The large amounts of sodium and water retained within the

body in the days immediately following operation, especially when coupled with concomitant potassium losses described in the last chapter, point to a major rearrangement of body fluid distribution. The disturbance of osmotic balance occasioned by the retention of large amounts of sodium, the preponderant extracellular cation, results in a shift of fluid from the cells into the extracellular space starting as a localised reaction in the injured region but inevitably spreading to involve undamaged areas. Thus Rosenthal and Tabor (1945) and Schilling et al. (1953) in injured animals, and Winfield et al. (1951) in post-operative patients, analysing the electrolyte content of injured muscle, concluded that the amount of sodium exceeded that expected from the amount of oedema fluid present, and, when taken in conjunction with the deficit of potassium in the injured muscle, all three groups of workers came to the conclusion that there had been an exchange of sodium for intracellular potassium, pointing to a change in cell membrane permeability following injury. This conclusion was borne out by the use of radioactive sodium in traumatized mice (Fox and Keston, 1945) and by the analysis of the sodium content of red blood cells in patients before and after major surgical operations (MacPhee, 1953).

But again, this exchange of sodium for intracellular potassium must only be a small part of the sodium retention following operation since the increased potassium output is over in a matter of 24 or 48 hours whereas the decreased sodium output continues for a number of days. LeQuesne and Lewis (1953) emphasized this dissociation in time between the potassium and

sodium responses to surgery in their detailed study of 21 male patients. They divided their patients into three groups, one of which received a constant sodium intake throughout, another of which received no sodium whatsoever for three days post-operatively, and the third of which received constant sodium with a large potassium supplement throughout. The group which received no sodium showed a primary water retention in the first 24 hours, as did the other two groups, showing that this was purely a water phenomenon and could be dissociated completely from sodium retention. In those groups receiving sodium there was also sodium retention in this period showing that the two phenomena were contemporary although not interdependent. Finally, LeQuesne and Lewis noted a period of late sodium retention which might coalesce with the period of early sodium retention, or might be separated from it by 24 hours of negative sodium balance. They also found that giving large supplements of potassium appeared to render the period of late sodium retention less both in duration and degree. These authors concluded that the primary water retention was the result of increased secretion of antidiuretic hormone (ADH) by the posterior pituitary, although their evidence on this point was purely circumstantial, and that the periods of sodium retention were probably partly or wholly due to a release of adrenal cortical hormone consequent on operation, renal haemodynamic factors probably contributing in part to the early sodium retention.

Perhaps the most noteworthy aspect of the sodium response to surgical trauma as opposed to the nitrogen and potassium responses

is the fact that the degree and duration of the sodium retention do not appear to bear any relation to the magnitude or nature of the surgical trauma. Thus it would seem that this fall in urinary sodium output, which is also the most consistent of the metabolic changes, is in fact primarily a result of operation per se, and not of altered food intake, tissue damage, loss of blood, etc., although all of these no doubt play a part in the response. It is also noteworthy that the sodium response was the only one which Moore and Ball (1952) could not duplicate in toto with their judicious mixture of starvation, immobilisation, and ACTH administration in healthy subjects.

However, in this, as in the preceding discussions of the alterations in nitrogen and potassium metabolism following trauma, the hormones of the adrenal cortex have been implicated. It is now therefore time to survey the part played by these ubiquitous hormones in the metabolic alterations following trauma.

5. THE ROLE OF THE ADRENAL CORTEX IN THE METABOLIC CHANGES FOLLOWING TRAUMA

The broad outlines of the effects of the hormones of the adrenal cortex on electrolyte metabolism were known by the end of the 1930's. Much of the work was carried out by observing the effects of administered adrenocortical extract in previously adrenalectomized animals (Silvette and Britton, 1933; Loeb et al. 1933; Harrop et al., 1933), by maintaining normal and adrenalectomized animals on diets containing extreme amounts of sodium and potassium and observing the results (Swanson and Smith, 1936; Swingle et al., 1937), and by studying the plasma electrolytes of patients with Addison's disease, long known to be due to hypofunction of the adrenal glands, and with Cushing's syndrome, suspected by analogy to be due to hyperfunction of the adrenal glands. The net result of this work was to show that the hormones of the adrenal cortex caused a retention of sodium and chloride within the body, while facilitating renal loss of potassium.

The first clear demonstration of a role of the adrenocortical hormones in protein metabolism occurred in 1940 when Long et al. administered adrenocortical extract and C-11-oxygenated corticosteroids to fasted normal, adrenalectomized and hypophysectomized rats and found both increased nitrogen excretion and liver glycogen deposition, suggesting a stimulation of gluconeogenesis from tissue protein. This interpretation has in general proved to be well justified, subsequent metabolic studies, such as those of Ingle et al. (1947b), showing negative nitrogen balances to be

produced if sufficiently large amounts of adrenocortical hormone were given. On the other hand, a number of observers (Forsham et al., 1948; Sprague et al., 1950) noticed that, in man, large doses of adrenocorticotrophic hormone (ACTH) or cortisone did not always induce negative nitrogen balances, and these, when they did occur, were of very variable magnitude.

As well as these facts emphasizing the protein catabolic effects of the adrenocortical hormones, however, evidence was later forthcoming that on occasion these hormones could apparently promote anabolism. Instance Ingle and Prestrud's work (1949) showing that the daily administration of 1-2 ml. of adrenocortical extract decreased the non-protein nitrogen excretion and increased the rate of growth of force-fed adrenalectomized rats compared with saline-treated controls or animals receiving large amounts of adrenocortical extract. It became apparent that small doses of hormone under the appropriate circumstances could promote protein anabolism, whereas large doses almost invariably stimulated protein catabolism, directly or indirectly, suggesting that these hormones were concerned with regulation of existing functions rather than with producing specific responses.

Experiments on hypophysectomized animals firmly established the fact that the anterior pituitary produced some trophic hormone which stimulated the adrenal cortex. Deane and Greep showed (1946) that the adrenal cortex atrophied after hypophysectomy; Ingle (1938) and Sayers et al. (1944) demonstrated that stress, which normally increased the size and decreased the concentrations of sudanophilic substances, cholesterol and

and ascorbic acid of the adrenal cortex, failed to influence the gland in the absence of the pituitary; a number of workers showed that the hypophysectomized animal, like its adrenalectomized counterpart, was extremely sensitive to a variety of non-specific stresses (Baird et al., 1933; Joseph et al., 1943; Tyslowitz and Astwood, 1942); and finally Baird et al. (1933) and Tyslowitz and Astwood (1942) showed that administration of adrenocortical extract increased the resistance of hypophysectomized rats to cold.

The trophic factor which stimulated the adrenal cortex was isolated as a homogenous protein from sheep (Li et al., 1943) and hog (Sayers et al., 1943) pituitary tissue. This adrenocorticotrophic hormone when highly purified, was found to restore the weight and histology of the adrenal glands of the hypophysectomized rat to a state indistinguishable from normal (Sayers and Sayers, 1948), to reproduce all the gross, histological and chemical changes which had been observed to occur in the adrenal cortex under a variety of conditions of stress (Sayers and Sayers, 1948), and, when administered to man, to produce all the metabolic changes ascribed to the hormones of the adrenal cortex, for example, retention of sodium (Conn et al., 1948) and fall in circulating eosinophils (Forsham et al., 1948). The question of the mode of regulation of pituitary ACTH will be considered later - for the present it will suffice to note that increase in adrenocortical activity would seem to be mediated by an increase in output of ACTH by the anterior pituitary.

That an increase in the urinary output of substances with

some of the properties of the adrenocortical hormones occurred after operation was first shown by Weil and Browne (1939), using the Selye-Shenker cold test (1938), i.e. measuring the mean survival time of adrenalectomized rats given known amounts of the extract under test. They interpreted this increase as a manifestation of increased adrenal activity which was part of a protective mechanism elicited as a response to a damaging stimulus.

That urine did, in fact, contain a substance, or substances, with some of the properties of the hormones of the adrenal cortex, had first been shown by Perla and Marmorsten-Gottesman (1931) who found that a benzene extract of normal human urine would increase the resistance of adrenalectomized rats to histamine. Then, in 1932, Grollman and Frior reported that benzene-soluble material from urine would prolong the life of adrenalectomized rats. This was followed by the discovery of Anderson et al. (1938) that a similar extract from the urine of patients with Cushing's syndrome was potent when assayed for its capacity to maintain life in the adrenalectomized rat whereas extracts of normal urine were inactive. Dorfman and co-workers, in a series of experiments published in 1943, showed that extracts of pooled normal human urine were possessed of the following biological activities in adrenalectomized rats - protection against low environmental temperatures, maintenance of life, increased muscle work performance, increased formation of liver glycogen, and protection against water intoxication, i.e. practically all the biological activities of extracts of the adrenal cortex known at that time. This suggested strongly that these substances were, in fact,

adrenocortical hormones, a suggestion further substantiated by Dorfman et al. (1944b) who were unable to find any comparable activity in urine extracts of seven patients with Addison's disease maintained on DCA and salt, although when maintained on adrenocortical extract some activity was detectable in most cases. These authors (1944a) were also unable to detect adrenocortical activity in the urine of monkeys following adrenalectomy, although substantial adrenocortical activity was detectable in the urine of most of those same monkeys when intact and after castration, showing that neither the ovaries nor the testes were the source of the urinary activity. They further made the interesting observation that, when the adrenalectomized monkeys were maintained on adrenocortical extract in place of DCA, urinary excretion of cortin-like material was again demonstrable.

Weil and Browne's original observation of increased "cortin-like" substances in human post-operative urine was confirmed in 1943 by Venning et al., using life-maintenance and growth of adrenalectomized rats as indices of adrenal activity, and again in 1944 by the same authors using the cold-protection and liver glycogen deposition tests in addition to the two already mentioned. They concluded in the latter paper that post-operative subjects excreted 3-30 times more cortin than did normal subjects and were able to recover in the urine 7-12% of large doses of administered adrenocortical extract. Reviewing the evidence for increased adrenal activity after operation, Shipley et al. (1946) commented on the lack of specificity of, and variation in quantitative results given by the biological methods then in use - the only

chemical method available being the almost equally non-specific Zimmermann method for 17-ketosteroids, the group of steroid metabolites known as the 17-ketosteroids being aptly described by one worker (Dorfman, 1944b) as "a metabolic waste basket which remains to be properly sorted and the contributory sources identified". Shipley et al. concluded, however, that the sum of the evidence for urinary output of adrenocortical hormones was convincing, as was the evidence that the output was increased after operation and other severe stresses, a conclusion which has proved to be accurate. Among their own results on patients undergoing surgical procedures, Shipley and colleagues included a patient of particular interest - one who had only a local anaesthetic but who nevertheless showed a marked post-operative increase in adrenal steroid hormone output, thus demonstrating that the increase in urinary steroids could not be attributed to the effects of general anaesthesia. These authors assumed that the increased excretion of adrenal steroids reflected increased secretion, thinking decreased tissue affinity or increased renal excretion to be unlikely, while conceding the possibility that decreased inactivation might be of some importance.

Paralleling the growth of knowledge concerning the metabolic effects of adrenal steroids was the weight of evidence accumulating that any non-specific stress, including surgical operation, produced the same metabolic effects, pointing to a stimulation of the adrenal cortex by all such stresses. The obvious conclusion to be drawn was that the metabolic changes occurring during stress were a direct consequence of the enhanced secretion of adreno-

-cortical steroids. It was Selye who (1936; 1940; 1941; 1946) gathered together all the facts concerning the body's reaction to stress of any kind and welded them into a whole, pointing out that the reaction was the same whatever the stress, that the adrenal cortex was implicated in every case, and elaborating his conceptions of the "alarm reaction" and the "general adaptation syndrome". Any non-specific stress was presumed to stimulate the anterior pituitary in some way to produce an increased amount of ACTH which in turn caused the adrenal glands to elaborate increased amounts of steroid hormones. The mechanism by which the anterior pituitary was stimulated was, and indeed still is, obscure. Sayers (1950) considered that a decrease in the level of cortical hormones in the body fluids acted as a stimulus to increased ACTH production. This theory was supported by many workers who showed that chronic overdosage with adrenocortical extract or DCA caused adrenal atrophy, and by Taylor et al. (1949) who claimed to be able to detect ACTH in the blood of untreated patients with adrenocortical insufficiency but not in the blood of normal subjects. This, however, appeared to be rather a sluggish homeostatic mechanism, requiring several days for its effect to be felt, whereas Gray and Munson (1951) demonstrated that after stress in the form of histamine, a release of ACTH occurred very rapidly - within ten seconds in fact. This rapidity of release would appear to point to a neural rather than a humoral mechanism, although there is some disagreement on this point. There is now a large body of evidence indicating that the stimulation of the anterior pituitary is via the hypo-

-thalamus (Uotila, 1940; Rauschkolb and Farrell, 1956a; Hume and Wittenstein, 1950).

It was Albright, however, who in 1943 first postulated the primary responsibility of the adrenocortical hormones for the metabolic changes following trauma, suggesting that increased secretion of adrenocortical hormones caused increased gluconeogenesis from protein which led to the observed increase in nitrogen excretion, a view later endorsed by Selye (1946). This view, based originally on the evidence of increased secretion of adrenal steroid hormones during stress, and the observation that certain of the metabolic responses to injury did not occur in the absence of the adrenal glands, and later substantiated by the finding that overdosage with ACTH or cortisone produced metabolic changes comparable to those following stress, seemed eminently reasonable and met with widespread acceptance. However, with further investigation it became increasingly apparent that this concept was an oversimplification and the brilliant studies of Ingle (1947a, 1947b, 1951a, 1951b) and Engel (1951, 1952, 1953) already referred to, showed incontrovertibly, in adrenalectomized animals, that although adrenocortical hormone must be present for certain of the changes under consideration to take place, the adrenal cortex was not itself directly responsible for these changes. Thus the adrenalectomized animal exhibited a normal metabolic response to injury if supplied with an adequate amount of adrenocortical hormone, which in itself did not produce overdosage effects. Ingle therefore maintained that the role of the adrenal cortex was a "permissive" or "supporting" one aimed at maintaining homeostasis rather than initiating the series of

events found to occur following injury. This latter concept of the role of the adrenal cortex being an indirect one was eventually accepted by Selye himself (1954) and many other workers.

It must be borne in mind that these early experiments on the actions of the hormones of the adrenal cortex were mainly carried out on adrenalectomized rats which can, unlike adrenalectomized patients, be maintained for a considerable time on sodium chloride alone without any steroid replacement. This, in itself, points to a different degree of dependence on the hormones of the adrenal cortex. In addition, crystalline steroids were rarely available and the adrenal hormones were usually administered in the form of crude adrenal gland extracts of varying potencies. And finally, methods of assaying the amounts of hormone excreted were fairly crude, non-specific, and mainly biological.

However, with the isolation of individual adrenal steroids and the elaboration of satisfactory methods for their syntheses in the years between 1936 and 1948, pure crystalline steroids became widely available, resulting in more precise methods for their estimation in biological fluids, and also removing many of the hazards consequent on the operation of total adrenalectomy by providing more exact means of steroid replacement therapy.

With the improved methods for estimation of the adrenal steroid hormones came incontrovertible evidence that the urinary output of adrenocortical hormones was greatly increased following major operations (Thorn et al., 1953; Cope and Hurlock, 1954; Tompsett and Smith, 1954; Moore et al., 1955; Reece et al., 1957), although an absence of such an increase following relatively

minor surgical procedures was frequently commented upon. The increases in urinary steroid excretion were seen to last for one to three days at the most, whereas the nitrogen and more particularly the sodium changes as already noted often lasted for as long as seven days. This led to some speculation as to whether or not these relatively short-lived increases in steroid excretion could be the cause of the longer lasting events. Relative to this point may be mentioned again the work of Moore and Ball (1952) who attempted to reproduce the metabolic effects of surgery in normal subjects by a judicious combination of short-term starvation, immobilisation and large doses of ACTH. They succeeded in reproducing all the usual metabolic changes, but the sodium retention lasted for a much shorter period, and was succeeded much sooner by a sodium diuresis than was the case after operation. However, it was widely realised that the increase in urinary excretion of adrenal hormones could be the result of one or both of two factors - an actual increase in secretion of hormones by the adrenal glands or a decrease in the rate of metabolism and removal of the hormones from the body.

In the meantime, yet another problem arose - that of aldosterone excretion. It had long been a puzzle why the amorphous fraction left after all the known crystalline steroids had been extracted from adrenal gland preparations was much more active in causing sodium retention and potassium excretion than were any of the crystalline steroids. This problem was resolved by two groups of workers almost simultaneously (Simpson et al., 1953; Mattox et al., 1953). They succeeded in isolating from the so-

-called "amorphous fraction" very small quantities of yet another crystalline steroid, subsequently named aldosterone, which was much more active in its effects on electrolyte metabolism than any of the known steroids. Owing to the fact that aldosterone secretion, unlike the secretion of the other adrenal steroids, was shown to be largely independent of anterior pituitary control and possibly, therefore, of the effects of stress (Cope and Llaurado, 1954; Gordon et al., 1954; Axelrad et al., 1954; Farrell et al., 1956; Venning et al., 1956), the question arose as to whether or not the excretion of aldosterone also rose post-operatively.

Aldosterone, not possessing a 17-hydroxyl group, would not have been measured by any of the methods for steroids containing this group so widely used, and being possessed of such a high specific activity, an increase in aldosterone excretion sufficient to account for all the electrolyte changes observed after operation would have been so small as to be undetectable by any of the methods for measuring total adrenal steroids. Nothing was therefore known about the post-operative excretion of aldosterone. Specific methods for measuring aldosterone were, and indeed still are, tedious and highly unsatisfactory. However, a number of workers using a variety of methods for measuring aldosterone - biological and physico-chemical; with or without preliminary chromatographic separation from the other urinary steroids; after acid hydrolysis or β-glucuronidase hydrolysis or no hydrolysis at all - have put forward evidence that the excretion of aldosterone, too, is increased after major surgical

operations (Llaurado, 1954; Llaurado, 1955; Zimmermann et al., 1956; Llaurado et al., 1956; Llaurado and Woodruff, 1957). It would seem, taking into account the variety of methods used and the lack of evidence to the contrary, that there was, in fact, a true increase in aldosterone excretion in the post-operative patient. It is of interest that Zimmermann (1956), considering why the output of a hormone not under ACTH control should increase following operation, postulated that, since a low sodium intake was known to increase aldosterone excretion (Luetscher and Axelrad, 1954), the lowering of serum sodium often found following surgery might well be the stimulus to increased aldosterone output, i.e. he thought that the aldosterone response was a consequence of the sodium response and not vice versa.

The question of whether the increase in total steroid excretion observed post-operatively was due to increased secretion by the adrenal gland or decreased rates of metabolism and disposal could obviously best be decided by investigation of the levels of circulating adrenal hormones, first in the peripheral blood and then in the adrenal venous blood itself. Following the elaboration of a satisfactory method for the estimation of 17-OHcorticosteroids in blood by Nelson and Samuels (1952), much work was done on the blood levels of these steroids in a variety of conditions. Since it was shown that hydrocortisone, which is measured by this method, is the principal steroid found in adrenal venous and peripheral blood (Sweat et al., 1953; Elman et al., 1955), modifications of this method have been used for practically all subsequent measurements of circulating adreno-

-cortical hormone. A large number of workers devoted their energies to measurements of blood 17-OHcorticosteroids before and after major surgical procedures (Moncrief et al., 1953; Sandberg et al., 1954; Franksson et al., 1954; Steenburg, 1954; Elman et al., 1955; Mittelman and Barker, 1956; Steenburg et al., 1956; Helmreich et al., 1957; Leftin et al., 1957), and found that in most cases there was a definite rise of fifty to several hundred per cent in the first 24 or 48 hours after the operation, usually followed by a fall to sub-normal values, the blood levels of 17-OHcorticosteroids returning finally to normal sometime within the 72 hours immediately following the operation. However, it must be noted that in a number of these authors' series, one or more cases occurred in which there was no rise in the blood steroid levels whatsoever following the operation (Sandberg et al., 1954; Tyler et al., 1954; Elman et al., 1955; Leftin et al., 1957). There was general agreement that minor surgical trauma such as muscle biopsy seldom produced a measurable increase in blood steroid levels but no comment has been made on this apparent lack of response in an admittedly small but definite number of patients undergoing major surgical procedures.

In general, the results were taken to imply that the metabolic events occurring after surgery were initiated by an increased blood level of adrenocortical hormones, Steenburg et al. (1956) maintaining that the evidence for the permissive role of the adrenocortical hormones did not preclude changes in the blood levels of these hormones and that these changes were responsible for the early post-operative metabolic alterations.

A number of the workers (Steenburg, 1954; Sandberg et al., 1954; Steenburg et al., 1956) made the interesting discovery that the post-operative rise in blood steroid levels was usually greater than the rise in response to injection, in the pre-operative period, of a dose of ACTH which was supposed to provoke maximum adrenal stimulation. Furthermore, the same dose of ACTH given in the immediate post-operative period, when the blood steroid levels were still raised, elicited a further rise in the levels. These results would seem to imply that the effects of ACTH and operation were superimposable and therefore due either to different mechanisms or to the fact that operation in some way increased the adrenal sensitivity to ACTH - an implication that would merit further investigation.

It was obvious that increased blood levels of adrenocortical steroids could result from increased rates of production by the adrenal glands or from decreased rates of metabolism, e.g. conjugation, hydrogenation, renal excretion. Because of the fact that the increased hormone levels after operation have never been shown to be preceded by decreased levels indicating increased tissue uptake, a measurable change in the tissue requirements of these hormones would appear to be ruled out as a cause of increased peripheral blood levels. An actual increased adrenal venous output of 17-OHcorticosteroids after operation in dogs was observed by Hume and Nelson (1954). By leaving the adrenal cannulae in situ they were able to obtain control values in the convalescent period. Hardy and Turner (1956) measured the adrenal venous output of 17-OHcorticosteroids in patients at operation

and found the values to be high but, in the absence of control values, this did not necessarily indicate a rise in adrenal output.

That the liver played an important part in the metabolism of the adrenal steroid hormones was shown in 1952 by Nelson and Harding who injected cortisone intravenously into dogs and measured the blood levels of 17-OHcorticosteroids. They found that the levels fell rapidly after the completion of the injection, being back to normal in sixty minutes, and that arterial and hepatic venous blood samples drawn simultaneously showed a marked difference across the liver. The time lag between the infusion of hydrocortisone or ACTH and the appearance of increased amounts of adrenal steroids in the urine had previously been shown and commented upon by Thorn et al. (1953) who had considered the time lag to be due to some form of metabolism of the hormones. Tomiza et al. (1954), injecting cortisone into intact and hepatectomized mice, found that the blood steroid levels rose in a similar manner in the two groups but stayed elevated for very much longer in the hepatectomized animals, pointing to a decrease in the rate of steroid metabolism as compared with the intact animals. The finding, in 1954, by Bongiovanni et al. that human blood contained B-glucuronidase-hydrolysable conjugates of the adrenal steroids virtually confirmed the role of the liver in the metabolism of the adrenal steroids, the liver being known to be the principal site of β-glucuronidase conjugation. Further confirmation came from the work of Reaven (1955) showing that adrenal steroids were inactivated when incubated with sliced rat or human liver under the appropriate conditions.

Some very interesting experiments were carried out in the same year by Hellman et al. (1954) using hydrocortisone labelled in ring A with C_{14}. These workers gave the labelled hydrocortisone either as a 0.25 mg. dose (i.e. a Physiological dose) or together with 100 mg. unlabelled hydrocortisone (i.e. a most unphysiological dose) to subjects with normal liver function and to an adrenalectomized woman both on and off maintenance hydrocortisone. In all cases the percentage of radioactivity excreted in the urine in the three days following the injection was the same and accounted for 80 per cent of the administered radioactivity. Since the same proportion of the dose was excreted whether a total of 0.25 mg. or 100.25 mg. of hydrocortisone was given, the rate of metabolism of the administered hormone must have been independent of the tissue requirements and there must have been no homeostatic mechanism brought into play to maintain the amount of hormone in the body at any fixed level. This conclusion was borne out by the case of the adrenalectomized woman who, although showing signs of adrenal insufficiency including alterations in renal function, nevertheless excreted the same proportion of the administered hormone as did the normals. These authors also showed that at the end of the infusion only 13 per cent of the blood radioactivity was present as unaltered hydrocortisone, demonstrating the rapidity of metabolism, and that only 0.05 per cent of the administered radioactivity was present in the expired carbon dioxide, indicating that the degradation of ring A represented only a very minor metabolic pathway.

Brown et al. (1954) gave intravenous hydrocortisone to

normal persons and patients with various liver diseases and found by measuring blood and urinary levels that the rate of disappearance of hydrocortisone was inversely proportional to the degree of liver damage as assessed by the bromsulphthalein excretion test. In 1955 Peterson et al. confirmed Hellmann's finding that infused hydrocortisone was metabolised at a constant rate whatever the dose given, and also found that in patients with cirrhosis of the liver the half-life of the infused hydrocortisone was perceptibly longer than in normal subjects and the rise in plasma conjugates was later and more prolonged, showing that liver damage definitely affected steroid metabolism.

It has been seen that the liver normally plays an important role in the metabolism of the adrenocortical hormones. What happens at operation ? It has long been known that a number of anaesthetics, particularly ether, caused a temporary decrease in liver function, but the reports of the effects of anaesthesia on the blood levels of free and conjugated 17-OHcorticosteroids were conflicting. Virtue et al. (1957) showed that varying kinds of general anaesthesia produced a rise in most patients whereas spinal anaesthesia in general did not. Four out of five of their patients who had known liver disease failed to show an appreciable rise after anaesthesia but showed a rise after operation. They concluded that impairment of liver metabolism could play little part in producing the increased blood levels of 17-OHcorticosteroids after surgery. Sandberg et al. (1954) on the other hand found that most patients showed a rise in blood steroid levels after anaesthesia, general or spinal. And on this evidence,

coupled with the fact that after giving intravenous hydrocortisone on operative and control days they could find no significant difference in the rate of its disappearance, they concluded that the increased blood levels found after operation were probably the result of a combination of increased production and decreased removal. They were careful to add that this did not imply that the metabolic response to surgery was due to these rises.

Several workers found that the increase in blood levels of conjugated steroids followed the same pattern as the increase in free steroids, but with a delay of several hours (Mittelman and Barker, 1956; Helmreich et al., 1957) although Hardy and Turner (1956) claimed to show a relatively greater increase in conjugates post-operatively, implying an increased, not decreased, rate of liver conjugation. Finally, Tyler et al. (1954), while measuring the post-operative increases in free blood steroids, also measured the liver function of their patients using the bromsulphthalein excretion test. They found that in general the greater the liver damage the greater the steroid rise.

It would seem apparent that the increased blood levels of adrenocortical found after major operations are in the main due to an increase in the rate of their secretion by the adrenal gland, although a decreased rate of conjugation by the liver may also be important, other modes of removal playing only a very minor role.

We are thus faced with a series of events occurring after any major surgical operation, which includes an increase in both

the blood and urine levels of free and conjugated adrenocortical steroid hormones. The important question is - Are these increases the cause of, or merely one of the consequences of, the body's response to the operation ? If man could exist without any adrenocortical hormone, endogenous or exogenous, this question could be quickly answered. Unfortunately, the nearest possible approach to this ideal situation occurs when the necessity arises to perform an operation on a patient who has already had his adrenal glands removed and is maintained on a constant dose of hormone. Fortunately or unfortunately, this situation is extremely rare. The next best set of circumstances for the purpose is the operation of bilateral adrenalectomy on a patient who is maintained before, during, and after the operation on a constant dose of adrenal hormone.

The first observations on steroid metabolism during bilateral adrenalectomy were made by Jenkins et al. (1953) who showed that, when patients undergoing adrenalectomy were infused with constant amounts of hydrocortisone on control and operative days, they showed no difference in the 17-OHcorticosteroid excretion pattern, thus showing no "gross utilisation" of steroids at operation. These results were interesting but led to no definite conclusion, as none of the other aspects of the metabolic response were measured, and comparison of the steroid excretion on two days is meaningless. The first detailed investigations into the metabolic response to adrenalectomy were made by Robson et al. (1955; 1956) who subjected several patients undergoing bilateral adrenalectomy in two stages to careful balance studies,

the patients being maintained for several days before and after the second stage on a constant daily dose of intramuscular cortisone acetate. The first stage was used as a control, the patients showing the usual nitrogen, sodium and chloride retention, increase in urinary potassium, and increase in urinary adrenocortical steroid output as measured by the somewhat unspecific acid stable formaldehydogenic steroid method of Tompsett and Smith (1954). The same events were seen to occur after the second stage, with the exception of the rise in steroid output. These results were confirmed by the work of Jepson et al. (1957) using the method of Norymberski (1953) for 17-ketogenic steroids as a measure of adrenocortical steroid output, and showed that if the events which follow surgery are indeed initiated by alterations in adrenocortical hormone metabolism, these alterations must be limited to the levels of circulating hormone and must not be reflected as changes in the amount of hormone excreted.

The only work on blood hormone levels after adrenalectomy has been done in dogs by Steenburg and Ganong (1955). These authors did not, however, investigate the blood levels at the actual operation of adrenalectomy but subjected the adrenalectomized dogs to a variety of trauma such as immobilization, anaesthesia, laparotomy, etc.. All these procedures were found to increase the blood steroid levels of the dogs given intravenous hydrocortisone but not of the dogs maintained on DCA implants alone, the authors concluding that the blood levels of free, and therefore active, steroids could be increased by extra-adrenal mechanisms, presumably delays in conjugation and excretion.

However, the number of dogs used was small, the rises in steroid levels very dubious in many cases, and the intravenous hydrocortisone only given for a short time before measurements were commenced - in short, the results obtained were somewhat inconclusive.

It would thus appear that the metabolic events which normally follow surgery can occur in the absence of a rise in the urinary output of adrenocortical steroids. The question of whether a rise in the circulating steroid level, not reflected by increased excretion, could occur and could account for the metabolic changes has not yet been answered satisfactorily.

THE PROBLEMS

6. THE PROBLEMS

Two outstanding problems remained to be solved regarding the metabolic events consequent on adrenalectomy.

It has been observed before (p. 34) that the methods normally used for measuring total urinary adrenal steroids either would not measure aldosterone or were not sensitive enough to measure a significant rise in aldosterone output. When relatively specific methods for the determination of aldosterone were applied to the urine of patients undergoing major surgical procedures not involving the adrenal glands, increased excretion was observed following operation, comparable to the increase observed in total adrenal hormone excretion. This increase, if it reflected a true increased rate of secretion of aldosterone by the adrenal glands, could well account for the electrolyte changes observed. However, it has been shown that following adrenalectomy no change can be demonstrated in the excretion of total adrenocortical hormones although the changes in electrolyte and nitrogen excretion occur as in any other major operation. The question that remains is whether or not there is an increase in available aldosterone following adrenalectomy - either from some extra-adrenal production source or from aldosterone remaining in circulation for some time after the removal of the second adrenal gland - either of which might account for the observed electrolyte changes, at least.

The second outstanding problem relates to the blood levels of adrenocortical hormone after adrenalectomy. It may be remembered that Steenburg et al. (1956) had firmly stated their view that the early post-operative changes were initiated by alterations in the blood levels of adrenocortical hormones. It was not impossible that even under

constant dosage with exogenous hormone, the blood levels at adrenalectomy might rise owing to decreased conjugation by the liver, thereby setting off the chain of events known as the metabolic response to surgery.

Previous research into the pattern of events following adrenalectomy had without exception been undertaken in patients given constant doses of intramuscular hormone. It was considered that dosage by such a route might well cause considerable fluctuations in the blood level of adrenocortical hormone. In an effort to keep the blood levels as constant as possible so that changes might be more readily observed, and to approach as near as possible to the physiological state, it was decided to maintain the patients on intravenous hydrocortisone given continuously for several days before and after the operation.

The two main problems, then, were to find out if there was a post-operative increase in aldosterone excretion, and to ascertain if there was any significant rise in the blood levels of adrenocortical hormones immediately following adrenalectomy; if either or both of these possibilities were shown to exist, the hormones of the adrenal cortex could conceivably still be implicated as causative factors in the metabolic response to surgical trauma.

CHOICE OF METHODS FOR STEROID ESTIMATIONS

7. CHOICE OF METHODS FOR STEROID ESTIMATIONS

1) Aldosterone

As early as 1950 Deming and Luetscher discovered that extracts of the urine of oedematous patients contained considerable amounts of a sodium-retaining material as measured by the retention of radioactive sodium in adrenalectomized rats according to the bio-assay method of Dorfman et al. (1947). The active material was presumed to be of adrenocortical origin and was thought at first to be desoxycorticosterone (DC) but Luetscher et al. (1952) dispelled this thought by showing the active material to be more polar than DC. With the isolation of crystalline aldosterone from the "amorphous fraction" of adrenocortical extract (Simpson et al., 1953; Mattox et al., 1953) and the realisation of its extreme potency in causing renal retention of sodium there arose the suggestion that the sodium -retaining material found in the urine might also be aldosterone or a closely allied substance. In a series of studies starting in 1953, Luetscher and co-workers (Luetscher and Johnson, 1953; Luetscher and Johnson, 1954; Luetscher et al., 1954) showed that the sodium-retaining substance found in considerable quantities in the urine of patients with nephrosis or heart failure closely resembled aldosterone in a variety of chromatographic, pharmacological and microchemical tests. The final identification of this urinary sodium-retaining factor as aldosterone was made by this same group of workers when they succeeded in isolating crystals of aldosterone from the urine of patients with congestive heart

failure and nephrosis (Luetscher et al., 1954, 1955, 1956). It was soon realised that the urine of normal persons also contained aldosterone although in much smaller amounts (Axelrad et al., 1955; Neher and Wettstein, 1955).

In the early studies lipid solvent extracts of urine were obtained, the crude extracts injected into adrenalectomized rats and the "sodium-retaining activity" measured. These mixtures were sometimes found to be toxic, leading to gross errors if sodium output was made the sole basis for assay. Estimation of the sodium:potassium ratio in the urine of adrenalectomized rats helped to avoid this error, but urine extracts still failed to give simple dose-response relationships, presumably because of the presence of interfering substances (Deming and Luetscher, 1950; Venning et al., 1955a). In a series of recovery experiments, the latter workers (Venning et al., 1955b) showed that the estimation of aldosterone added to urine was subject to a considerable degree of error if crude extracts were used. For this reason most investigators employed some method of fractionation of the urine extracts. Chloroform was the extracting solvent most commonly employed, and pigments, phenolic substances, and other interfering material were removed by washing the chloroform extracts with dilute alkali and water (Axelrad et al., 1955). Aldosterone was found to be easily destroyed by strong adsorbents but paper chromatography was found to give promising results as not only did it allow further separation of interfering material but it permitted a definite identification of the biologically-active material. Because of the non-specific nature of the

physicochemical tests available, an efficient method of separation of the various adrenal steroid hormones was necessary before the bio-assay results could be attributed to aldosterone and aldosterone alone.

The chromatographic system most widely used for the separation of crude urine extracts was the toluene/propylene glycol system introduced by Burton et al. (1951) which had a high capacity useful when handling large quantities of urine extract. The only drawback to this system was its comparative slowness, complete separation of the urinary corticosteroids taking up to 72 hours. When a series of active urine extracts were separated in this system an average of 75% of the initial sodium-retaining activity was recovered in the aldosterone fraction (Luetscher and Johnson, 1954). Serial assays of urine extracts during repeated chromatography showed progressive losses which were, however, small if reasonable care was taken (Venning et al., 1955b). A series of chromatographic systems in which separation of the main adrenocortical steroids was effected in a matter of hours was elaborated by Bush (1952) and proved to be of great value. These systems, however, had comparatively low capacities and a preliminary partial separation of the aldosterone-containing fraction on the Zaffaroni system, followed by complete separation of aldosterone from cortisone on one of the Bush systems, was found to be the best way of dealing with the large amounts of material present in chloroform extracts of urine (Neher and Wettstein, 1955).

Various bio-assay methods have been used by most workers

for the estimation of sodium-retaining activity in urine. Dorfman et al. (1947) and Dorfman (1949) demonstrated that as little as 1.0 µg of DC could be detected in adrenalectomized rats by measurement of the excretion of radioactive sodium. Other bio-assay methods based on sodium retention in adrenalectomized rats were elaborated by Kagawa et al. (1952), Marcus et al. (1952) and Singer and Venning (1953). Rather larger doses of DC were found, in addition, to increase potassium excretion and Johnson (1954) showed that more precise estimation of the dose of DC was possible if the urinary potassium:sodium ratio were measured than if only the sodium output of adrenalectomized rats was studied, a fact also utilised by Simpson and Tait (1952) in their bio-assay method using the ratio of Na^{24} to K^{42}. Both these latter sets of workers found that plotting the urinary potassium: sodium ratio against the logarithm of the dose of DC gave the most consistent results. However it was evident that whatever methods of bio-assay and of controlling electrolyte intake in the test animals were adopted, a considerable variation in electrolyte excretion persisted both from day to day and from animal to animal necessitating the use of large numbers of animals per assay. Also the ratio of aldosterone activity to DC activity varied widely in the different methods of bio-assay, Simpson and Tait reporting a ratio as high as 120, Johnson one as low as 30.

The quantity of biologically active material extracted from urine by chloroform was found to depend to a great extent on the method of extraction, only a very small amount of

aldosterone being found to be present in extracts of neutral urine (Cope and Llaurado, 1954; Axelrad et al., 1955). The yield of aldosterone was found to be greatly increased if the urine was first treated with strong acid or incubated with β-glucuronidase. It was found that if the urine was extracted immediately after acidifying to pH 1.0 or 1.5 with concentrated hydrochloric acid a considerable and reproducible yield was obtained from urines with a high aldosterone content (Venning et al., 1955b). Since these workers found that the recovery of free aldosterone added to urine was not altered by acidification, they assumed that endogenous aldosterone was freed from more polar conjugates at the acid pH. These workers also found that even larger yields of aldosterone were obtained if the urine was left to stand at pH 1 for 24-48 hours before being extracted, the increased yield far outweighing the minor losses caused by exposure to strong acid. These results were confirmed by Simpson et al. (1954), who demonstrated that aldosterone could be recovered largely unchanged after standing for 20 hours at pH 1. Treatment with β-glucuronidase was shown to allow extraction of somewhat smaller additional quantities of aldosterone from urine (Axelrad et al., 1955; Venning et al., 1955a and 1955b). This advantage was, however, found to be offset by the unduly large quantities of other material liberated by the β-glucuronidase which complicated the subsequent chromatography.

The drawbacks of the various bio-assay procedures for estimating the aldosterone content of urine were all too apparent but when the work incorporated in this thesis was started, no

physicochemical method applicable to the estimation of aldosterone, which could be regarded as being at all satisfactory, was available. It was true that a number of the non-specific reactions used to detect the other adrenal steroid hormones, such as the reduction of tetrazolium salts and ultra-violet absorbtion, would also measure aldosterone, but the amounts of aldosterone to be measured were so very small compared with the amounts of the other hormones likely to be present that very careful separation would obviously first have to be carried out. And then the measurement of such very small quantities of aldosterone as are found in the urine of normal persons presented intrinsic difficulties even supposing such a careful separation had been effected. Luetscher and Johnson (1954) showed that the ultra-violet absorbtion or tetrazolium reduction in the neighbourhood of aldosterone on a single chromatogram of urine extract bore no consistent relation to the sodium-retaining activity. Even after rechromatography in a different system the results obtained with these methods of estimation did not correspond systematically with the quantities of aldosterone found on bio-assay. The most promising physicochemical method was that of Neher and Wettstein (1955) who used two different chromatographic systems and estimated the aldosterone thus separated by visual comparison of the spots obtained by ultra-violet absorption, blue tetrazolium reduction, and sodium hydroxide fluorescence with the spots given by known amounts of hydrocortisone chromatographed simultaneously. While Neher and Wettstein's method contained many desirable features - extraction of the urine after 24 hours

of acid hydrolysis; the use of the Zaffaroni toluene/propylene glycol chromatography system for separation of the aldosterone-cortisone fraction from the rest of the lipid material; the use of the Bush C chromatography system for the complete separation of aldosterone from cortisone - it seemed that the visual estimation of the separated aldosterone merely by comparison with known amounts of hydrocortisone chromatographed simultaneously was unsatisfactory in the extreme. It was therefore decided for the work described in this thesis to extract and separate the urinary aldosterone by this method but then to elute the aldosterone-containing area from the second chromatogram - the area being readily located using reference strips of hydrocortisone which runs at the same rate as aldosterone in this system - and assay the aldosterone content by bio-assay in adrenalectomized rats.

Preservatives having been found to be of doubtful value, the urine was collected without preservatives and, when not extracted immediately, stored below $0^{\circ}C$ at which temperature it has been shown to be stable for several months.

The bio-assay method of Johnson (1954) was chosen as employing comparatively few rats and not employing radioactive sodium or potassium, whilst being reasonably satisfactory statistically. No aldosterone being obtainable in the first instance, a standard curve was constructed using DCA, a linear/log. dose response curve being obtained with doses of DCA ranging from 2 to 20 µg. per rat. The urine fractions subsequently assayed were adjusted so that the response fell within these

limits. Johnson, in a series of experiments with pure aldosterone, found it to be 36.5 times as potent as DCA in this method of assay. In the absence of any pure aldosterone with which to conduct similar experiments, Johnson's conversion factor of 36.5 was temporarily adopted. When eventually a very small quantity of aldosterone did become available, several assays were carried out with it and gave a mean conversion factor of 33.6. Johnson's figure of 36.5 obtained in a much larger series of assays was therefore adhered to as being applicable to the assay in our hands and the amounts of aldosterone reported in this thesis were obtained by dividing the estimated amount of DCA by 36.5.

Estimation of the urinary excretion of aldosterone in 6 normal persons gave values which ranged from 1.1 to 3.5 µg./24 hours, results which agreed reasonably well with normal values obtained with similar methods by Venning et al. (1956), who quote a range of 1 to 6 µg./24 hours, and by Luetscher and Axelrad (1954) who quote a range of 1.8 to 3.5 µg./24 hours. Later workers, using purely physicochemical techniques, have obtained somewhat higher normal values, the discrepancy being due in all probability to low bio-assay results caused by losses of active material, the presence of interfering substances and limited response, the non-specific nature of the physicochemical methods leading on the other hand to unduly high estimates. Indeed Luetscher et al. (1954, 1956) have twice isolated substantially larger amounts of pure aldosterone from pooled extracts of human urine than were obtained by bio-assay.

Although this combination of a reasonably extensive method

of extraction and chromatographic separation of urine, with the biological estimation thought to be necessary for the small amounts of aldosterone expected to be present in the pre-operative urines of the patients studied, was thought to be the best available, its shortcomings were fully realised. Indeed the very small percentages of aldosterone added to urine recoverable by any of the available methods, percentages in the range 15-30%, are eloquent of these shortcomings. However, the aldosterone content of the urines in the last four cases reported in this thesis was estimated independently by the physicochemical method of Nowaczynski et al. (1956). Despite the fact that higher results were obtained with the purely physicochemical method, the results obtained by the two methods paralleled each other in that when a urine extract gave a high result in one method it gave a high result in the other method; similarly with low results. This was taken to indicate that the biological method, although unsatisfactory, was adequate for the purpose for which it was required.

2) Total Urinary Steroids

When it is considered that in 1952 Pincus et al. had identified 35 different steroids in human urine, and that a number of others have since been added to this formidable list, it is obvious that the choice of method for estimation of urinary steroids must depend on the purpose in mind. For the purposes of this research a method was required which would

provide a good index of the state of activity of the adrenal gland before its removal from the body, and which would also measure a consistent fraction of the metabolites of the administered cortisone or hydrocortisone on which the patients were maintained during operation and following the complete removal of the adrenal glands. It was immediately apparent that although a number of specific but somewhat laborious and time-consuming methods employing extensive fractionation of the urine by paper-chromatographic or other means were available for the estimation of individual steroids, no one of these would suffice as giving sufficient information as to the activity of the adrenal gland as a whole. Besides which, the necessity for one person to do daily urinary and blood steroid estimations on each patient studied, together with electrolyte determinations, etc., meant that the method chosen had to be relatively simple and require as short a time as possible for its completion. These requirements made inevitable the sacrifice of a certain amount of specificity in the search for simplicity, and a reasonable compromise was therefore sought among the methods based on more or less specific group reactions.

The C_{21} steroids are acknowledged to be the corticosteroids which best reflect adrenal activity and therefore methods determining this group were considered. This ruled out all methods for estimating 17-ketosteroids, a group of steroids which is known to arise largely from the androgenic steroids of the adrenal cortex and which has the additional disadvantage of arising in the male partly from the testicular hormones. Three main groups

of methods presented themselves for consideration: (1) those based on the determination of formaldehydogenic or reducing chromogens in crude extracts and measuring mainly those steroids with a glycol or α-ketol side chain; (2) those based on the Porter-Silber reaction (1950) for steroids with dihydroxyacetone side chains; (3) those based on the procedure introduced by Norymberski (1952) whereby the urinary "17-ketogenic" steroids were subjected to oxidative degredation and the resulting 17-ketosteroids measured.

The methods of the first group, although relatively quick and simple to perform and supplying much useful clinical information as to the state of activity of the adrenal glands, have been subject to much criticism from academic biochemists on the grounds of lack of specificity. The adrenocortical hormone metabolites in the urine have long been known to be present mainly as sulphate and glucuronide conjugates, and recent work has shown that 87% of infused radioactive hydrocortisone was excreted as some form of conjugate whereas only 4% was excreted as unconjugated metabolites (Petersen et al., 1955; Hellmann et al., 1956). It was at the methods employed for hydrolyzing these conjugates that the criticism was mainly aimed. Strong acid hydrolysis was the method most commonly used, the urines frequently being acidified to pH 1 and even boiled for some considerable time as in the method of Tompsett (1953). It soon became evident, however, that whereas some adrenocortical steroids such as DC were completely stable to such procedures, others, most noticeably cortisone, emphatically were not, recoveries of

this steroid being inconsistent and often as low as 30% (Marrian et al., 1953). Marrian et al. concluded that the formaldehydogenic steroids measured by this and similar methods consisted largely of artifacts formed during the vigorous acid hydrolysis. Enzymatic hydrolysis with β-glucuronidase gave divergent results, partly due to the use of different enzyme preparations and partly because of the presence of various enzyme inhibitors, activators, and differing concentrations of substrate present in the urine (see, for example, Dyrenfurth and Venning, 1957; Mason, 1954; Horwitt and Altschul, 1953). Although the method of Tompsett cited above had proved to be of considerable value and had been used in previous studies on the metabolic response to surgery (Robson et al., 1956) where it had been shown to reflect the increased adrenocortical excretion found after operation, it was felt that the lack of specificity was a grave disadvantage and it was therefore superseded in all cases but the first studied, by a method taken from the second group, i.e. one based on the Porter-Silber reaction.

This reaction, while measuring all steroids with a 17-hydroxyl group including cortisone, hydrocortisone, and their tetrahydro derivatives which are known to be the major urinary corticosteroid metabolites, does not measure several of the other adrenocortical hormones of importance; in particular it does not measure aldosterone, corticosterone, and their metabolites. This was not felt to be a major drawback in these investigations as aldosterone was being measured separately and the biological significance of corticosterone has yet to be established with

certainty. Previous experiments had shown that a distinct rise in total urinary 17-OH corticosteroids occurred following ACTH administration in man (Thorn et al., 1953; Liddle et al., 1954) suggesting that this group of steroids formed an adequate index of adrenocortical activity. Methods based on this reaction had the additional advantage that if blood steroids were to be measured concurrently it would obviously be preferable to measure comparable groups of steroids in blood and urine, and the majority of the existing methods for estimation of blood steroids rested on the Porter-Silber reaction.

Various modifications of the original method as applied to urine (Reddy et al., 1952) were in existence, the differences lying mainly in the methods used for partial purification of the butanol extracts of urine which contained both free and conjugated corticosteroids as well as variable amounts of other chromogens. The method of Forsham et al. (1955) employing immediate extraction following acid hydrolysis was chosen as being both satisfactory and not unduly time-consuming.

Since preservatives are of doubtful value and the urinary content of 17-OH corticosteroids has been found to diminish quite rapidly at room temperature, the urines were collected without preservatives and refrigerated as soon as passed. Because 24 hour urine collections were made in all cases the diurnal variation in excretion of these steroids did not have to be taken into account. Care was taken to ensure that the patients received no form of medication liable to interfere with this method, such as sulphonamides, ascorbic acid, quinine, etc..

A group of 15 normal members of the hospital staff gave a mean daily excretion of 17-OH corticosteroids by this method of 6.77 ± 2.37mg./24 hours, the range extending from 4.3 to 10.5mg./24 hours. This range of normal values agreed satisfactorily with ranges obtained by other workers using similar methods, e.g. Forsham et al., 1955 (4-14 mg./24 hours); Silber and Porter, 1954 (3.0-10.3mg./24 hours); Leftin et al., 1957 (0-10.0mg./24 hours). 8 patients with untreated Addison's disease or hypothalamic lesions gave values ranging from 0.00-2.99mg./24 hours, and one patient with untreated Cushing's syndrome showed an excretion of 15.74mg./24 hours. No recovery experiments were done as no pure steroid conjugates were available.

Although the percentage of total urinary steroids excreted in the free form is so small under normal conditions as to be of little significance, it was thought that this percentage might show some significant alteration following operation when steroid metabolism in general is altered. The method of Silber and Porter (1954) for the determination of free urinary 17-OH corticosteroids was therefore adopted and a range of normal values of from 0.24-1.17 mg./24 hours with a mean of 0.49 ± 0.26 was obtained in 16 normal individuals, these values agreeing reasonably well with Silber and Porter's mean normal excretion value of 0.37 ± 0.18 mg./24 hours.

The steroid content of a number of the urines reported in this investigation was also measured by a method from the third group, namely the method of Moxham and Nabarro (1956) for 17-ketogenic steroids. Since this method has no striking

advantage over the 17-OH corticosteroid method, and a normal range of values has not yet been established for this laboratory, and since the results gained by the two methods have been essentially the same, only the urinary 17-OH corticosteroid values have been presented in this thesis.

3) Free Blood Steroids

Most of the present-day methods used in estimating the adrenal hormones circulating in the peripheral blood are based on the Porter-Silber reaction which was first applied to plasma by Nelson and Samuels in 1952. Since hydrocortisone has been shown to be the predominant steroid hormone present in human peripheral blood (Sweat et al., 1953), and since under favourable conditions the plasma Porter-Silber chromogens have been shown to consist almost entirely of hydrocortisone (Petersen et al., 1957), these methods have proved, in the main, to be satisfactory. Approximately half of the plasma adrenocortical steroids have been shown to be present in the form of β-glucuronide conjugates (Bongiovanni et al., 1954) but since these glucuronide conjugates are apparently devoid of any biological activity they are thought to represent the first stage in the metabolism of the free hormones. Preliminary treatment of the plasma samples has been shown to be necessary to remove other substances present in the lipid solvent extracts which interfere with the subsequent colour reaction. The original Nelson and Samuels method, together with

most of the subsequent modifications, utilised column chromatography , usually on Florisil, for the preliminary purification of the plasma. However, since Florisil is relatively difficult to obtain in this country and since variable results were obtained with the small amount of Florisil to hand, it was decided to use, if possible, a method which avoided the use of column chromatography. The time factor, all estimations being performed by one person, precluded the use of some elaborate but extremely elegant methods of separation such as that of Weichselbaum and Margraf (1955). However a relatively simple method, as yet unpublished, was found to be in use (O'Donnell, 1957).

This method replaces column chromatography with extensive solvent washing of the plasma which has been found to be effective in removing interfering substances. In common with the other methods in current use, this method required heparinized plasma, citrated blood having been found to give opalescent solutions on occasion, and employed the Allen colour correction (Allen, 1950). Despite these precautions, occasional plasma samples were encountered which did not display a maximum colour density at 410 microns and these samples were consequently discarded. Other workers including Eik-nes et al., (1953) have encountered similar difficulties for which no explanation is forthcoming. Because of the scanty data available regarding the modified method of O'Donnell it was thought necessary to undertake a short investigation of the accuracy of the method.

Plasma samples taken from 11 normal members of staff

Table 1.

17-OH corticosteroid content of plasma µg/10 ml.	Amount of hydrocortisone Added µg.	Estimated µg.	% Recovery
0.82	1.00	1.48	66.0
0.82	1.00	1.53	71.0
0.94	1.00	1.69	75.0
0.94	1.00	1.62	68.0
1.22	2.00	2.68	73.0
1.22	2.00	2.67	72.5
1.15	2.00	2.68	76.5
1.15	2.00	2.76	80.5
0.82	2.00	2.78	98.0
0.82	2.00	2.62	90.0
2.32	2.00	4.09	88.5
2.32	2.00	3.91	79.5
1.38	4.00	4.34	74.0
1.38	4.00	4.24	71.5
0.85	4.00	4.33	87.0
0.85	4.00	4.21	84.0
	Mean of 16 estimations		78.4

between the hours of 9 a.m. and 11 a.m. were estimated in duplicate and ranged from 6.0 to 23.2 µg./24 hours with a mean value of 12.6 ± 4.6 µg./24 hours. These values agreed reasonably well with values obtained by other workers using methods incorporating column chromatography, e.g. Silber and Porter, 1954, (6-25 µg./24 hours), Bayliss and Steinbeck, 1953, (3-16 µg./24 hours), Jailer et al., 1955, (4-32 µg./24 hours). One patient suffering from untreated Cushing's syndrome showed a plasma level of 36.3 µg./24 hours. To test the reproducibility of the method a series of recovery experiments was carried out in which known amounts of hydrocortisone varying from 1 to 4 ug. were added to plasma samples whose 17-OH corticosteroid content was measured simultaneously in duplicate. The results are shown in Table I. The amounts of hydrocortisone added were those expected to be encountered in the subsequent experiments. The percentage recovery of the added hydrocortisone was found to range from 68.0% to 98.0% with a mean recovery of 78.4%. The figures, while not being particularly good, compare satisfactorily with those obtained by other workers such as Bayliss and Steinbeck (1953) who obtained recoveries of added hydrocortisone varying from 68.0% to 93.0%. Variations in the value of normal plasma samples estimated in duplicate were found to vary by from 0.0 to 3.2 µg./100 ml. plasma.

From these figures it was evident that the method although not entirely satisfactory was of sufficient accuracy for the purpose intended and it was therefore adopted.

METHODS

8. METHODS

Sodium: Eel Flame Photometer.

Potassium: Eel Flame Photometer.

Nitrogen: Micro-Kjeldahl.

Free 17-OH Corticosteroids in Urine:

Method of Silber and Porter (1954).

Total 17-OH Corticosteroids in Urine:

Forsham's modification (1955) of the method of Reddy, Jenkins and Thorn (1952).

Free 17-OH Corticosteroids in Plasma:

Unpublished modification (O'Donnell) of the method of Silber and Porter (1954).

Reagents:

A.R. Anhydrous sodium sulphate.

Carbon tetrachloride
Petroleum ether (b.p. 40-60°C)
Methylene chloride

Purified by washing three times with 0.1 volume of distilled water, drying over sodium sulphate, and distilling. Methylene chloride was distilled immediately before use.

Sodium hydroxide 0.1N.

Sulphuric acid 62% v/v.

Phenylhydrazine hydrochloride A.R.:- Purified by recrystallisation from acetone.

95% Ethyl alcohol:- Purified by distillation over m-phenylenediamine dihydrochloride.

Alcoholic phenylhydrazine sulphuric acid reagent:- 6.5mg. of Phenylhydrazine hydrochloride were dissolved in a solution consisting of 10 ml. 62% sulphuric acid and 5 ml. 95% ethyl alcohol. This reagent was made up every day.

Procedure:

Heparinized blood samples were centrifuged and the plasma separated within 20 minutes of withdrawal of the blood. Aliquots of the plasma were made up to 10 ml. with distilled water, shaken for 30 seconds with 10 ml. of carbon tetrachloride, centrifuged, and the carbon tetrachloride layer removed by means of a syringe and a long blunt needle. This washing with carbon tetrachloride was pepeated once. The plasma samples were then shaken for 30 seconds with 10 ml. of petroleum ether, centrifuged, and the petroleum ether layer removed with a syringe. The washing with petroleum ether was repeated once. The plasma samples were then shaken for 30 seconds with 10 ml. of methylene chloride, centrifuged, and the methylene chloride layer separated. The plasma samples were extracted twice more with 10 ml. portions of methylene chloride, the extracts combined and washed by shaking with 2 ml. of 0.1N sodium hydroxide for 45 seconds. The aqueous sodium hydroxide layers were removed by means of a syringe and the residual extracts dried by filtering through anhydrous sodium sulphate. The extracts were then taken to dryness in a water bath at 40°C under a stream of air. The residues were dissolved in 4 ml. of methylene chloride, transferred to smaller tubes and taken to dryness as before. To

each of the final residues from the extracts, 0.6 ml. of the alcoholic phenylhydrazine sulphuric reagent was added, and the stoppered tubes were heated to 60°C for 30 minutes in a thermostatically controlled water bath together with a blank solution containing only 0.6 ml. of the alcoholic phenylhydrazine sulphuric reagent. At the end of this period the optical density of the solutions was determined against the blank at 370, 410, and 450 mμ in a Unicam S.P. 500 spectrophotometer with a microcell attachment, using 2mm. cells.

$$\text{Corrected optical density (C.O.D.)} = \text{Optical density (O.D.)}_{410} - \frac{\text{O.D.}_{370} + \text{O.D.}_{450}}{2}$$

A standard graph was obtained by taking alcoholic solutions containing 0.1-5 μg. pure hydrocortisone to dryness below 40°C, dissolving in 0.6 ml. of the alcoholic phenylhydrazine sulphuric acid reagent, and carrying out the colour reaction as above. The results for plasma 17-OH corticosteroids were expressed as μg. hydrocortisone.

Aldosterone

The urines were hydrolysed, extracted, and subjected to paper chromatography according to the method of Neher and Wettstein (1955). The aldosterone content of the extracts was estimated by the bio-assay method of Johnson (1954) using adrenalectomized rats.

PROCEDURE

9. PROCEDURE

With the exception of the day of operation and the first two or three days thereafter, all the patients studied received from the dietetic kitchen of the hospital a diet constant for each patient and containing calories within the range 1000 to 2000. The basic diet contained approximately 0.5g. sodium chloride (8.6m.Eq. sodium) and 1.2-2.0g. potassium (30-50m.Eq.) as well as a reasonably constant amount of protein. The greater part of the potassium was derived from canned fruit juice and each patient received a fixed daily allowance from a single batch, the potassium content of each batch of juice being first estimated. This was found to be necessary because, although the potassium content was found to be constant in any one batch of fruit juice, it varied considerably from batch to batch. The actual composition of the diets was calculated from tables but the results were checked by chemical analysis of at least one duplicate diet for each patient. The diets in the first three cases studied were supplemented with 4.5g. sodium chloride (77m.Eq. sodium) per day given by mouth as sachets. In the later cases who were being given intravenous hydrocortisone, the amount of sodium retained was found to be considerable and so the sodium supplements in these cases were reduced to 2g. sodium chloride (33m.Eq. sodium). On the day of operation no food was eaten but a slow intravenous infusion of 0.9 per cent sodium chloride was given to ensure an intake of 77m.Eq. sodium. On the first two or three post-operative days the intakes of

potassium and of nitrogen were estimated from the actual amount of the diet consumed, and the sodium intake of 77m.Eq. was maintained either by infusion or by oral administration. Thereafter the basic diet, supplemented by the relevant amount of sodium chloride, was once more given. In no case was it found necessary to give a blood transfusion either at, or following, the operation.

The patients who were investigated primarily from the point of view of aldosterone excretion were given 50 mg. of cortisone acetate three times per day by intramuscular injection. The cortisone administration was begun at least five days before the operation in order to suppress the endogenous secretion of adrenocortical hormones as completely as possible, and to ensure that the patients were in sodium, potassium, and nitrogen equilibrium for some days before the operation. This dose of cortisone was found to be quite sufficient to ensure uneventful recovery from adrenalectomy and subsequent uncomplicated convalescence.

The patients in whom the blood levels of 17-OH corticosteroids were being studied were given hydrocortisone by continuous intravenous infusion throughout the 24 hours. The first patient was given 200 mg. of hydrocortisone per 24 hours but as this dosage level led to considerable sodium retention with consequent oedema, the amount of hydrocortisone infused per 24 hours was reduced to 150 mg. in the three subsequent patients. The hydrocortisone was dissolved in 1800ml. of 6% dextrose and the constancy of the rate of infusion was

maintained as far as possible by frequent checks on the rate of the drip and by the use of a polyethylene catheter inserted into the superior vena cava to ensure a continuous free flow. The caval catheter was inserted, and the hydrocortisone infusion was started at least 24 hours before the first blood sample was taken as it had been found that the blood levels of 17-OH corticosteroids fluctuated considerably in the first 24 hours following the start of the infusion, thereafter settling down to reasonably constant levels.

No attempt was made to collect faeces passed during the period of observation since it has been shown that, unless gastro-intestinal lesions are present, there is no great variation in the amounts of the relevant substances excreted by this route. In addition, no stools are normally passed in the immediate post-operative period, a fact which greatly simplifies the interpretation of the results.

The urine collections in all of the patients were made in periods of 24 hours, the urine being stored in a refrigerator below 5°C as soon as passed. The total steroid content of the urines was estimated in duplicate immediately following the end of a collection, the other chemical analysis being carried out as soon as possible. The urine aliquots reserved for aldosterone estimation were hydrolysed, extracted, and chromatographed immediately. The dry purified extracts were then stored in a deep freeze below 0°C until it was possible to subject them to bio-assay - in no case was an extract stored for more than a few weeks, the majority being assayed within several days. It

has been shown that such extracts are stable below 0°C for several months. The 24 hour urine collections were in all cases timed so that the operation coincided with the beginning of a collection.

Blood samples for 17-OH corticosteroid estimation were withdrawn from the patients at 8 hourly intervals, the samples always being taken at the same time of day so that any effects of diurnal variation in the secretion of endogenous hormone could be taken into account. Care was taken to withdraw the blood from a vein as far removed from the site of entry of the catheter as possible. The plasma was separated from the heparinized blood within 20 minutes of withdrawal from the patient and was stored in a deep freeze below 0°C until it was possible to measure the steroid content, which was usually within several days. All the steroid estimates were done in duplicate. Additional blood samples were withdrawn either immediately preceding or immediately following the induction of general anaesthesia, and at varying time intervals in the hours immediately following the completion of the adrenalectomy. All patients underwent the usual pre-operative medication.

RESULTS

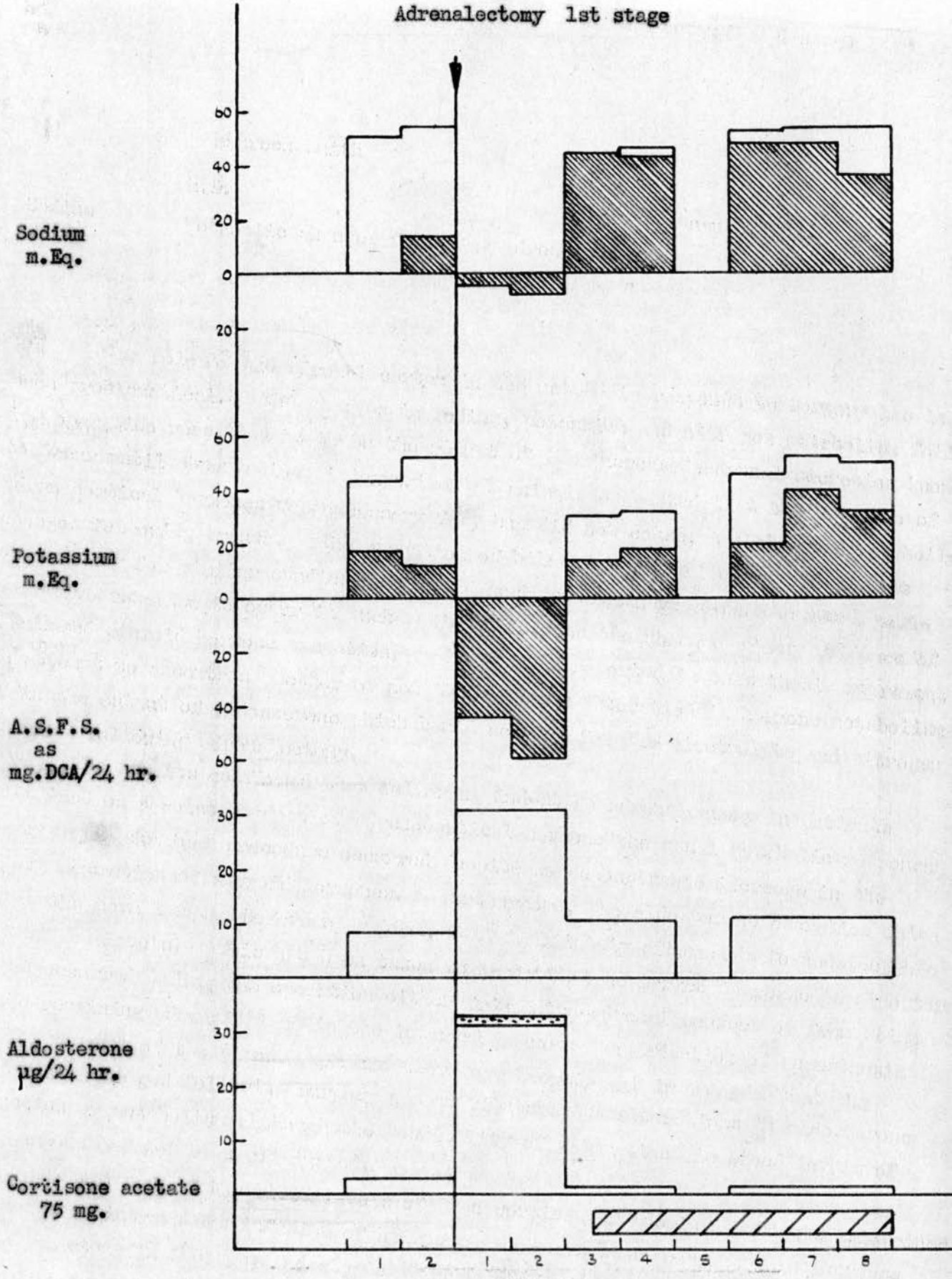


FIG. 1. D.G. Sodium and potassium balances and A.S.F.S. and aldosterone excretion before and after the 1st stage of a bilateral adrenalectomy.

RESULTS

The investigations fall into two parts. The first part deals with the excretion of aldosterone following adrenalectomy. The second part is concerned with the effects of adrenalectomy on the blood levels of free 17-OH corticosteroids.

1. ALDOSTERONE EXCRETION FOLLOWING ADRENALECTOMY

Because of the recognised unsatisfactory nature of the methods for measuring aldosterone in urine it seemed imperative to ascertain whether or not the method used was capable of detecting the increase in aldosterone excretion known to occur following major surgical procedures in patients with intact adrenal glands. The operation most suitable for the purpose of a control as regards duration and magnitude of trauma was obviously a first-stage adrenalectomy. The first case studied was therefore that of a patient (D.G.) undergoing a combined ovariectomy and first-stage adrenalectomy for carcinoma of the breast. It was planned to follow this patient also through the second stage of the adrenalectomy nine days later but unfortunately this was not possible as she died soon after the completion of the second operation. The results obtained following the first stage of the adrenalectomy are shown in Fig. 1. The data on sodium, potassium and nitrogen are presented throughout according to the method of Moore and Ball (1952), in which the intake of each constituent is

charted upwards from the zero line and the urinary output is charted downwards from the intake, black areas above and below the zero line thus representing accumulation and loss respectively. In the immediate post-operative period there was a normal metabolic response in this patient with decreased excretion of sodium leading to a marked positive balance for several days, increased excretion of potassium on the two immediate post-operative days, and a threefold rise in the urinary output of acid-stable formaldehydogenic steroids on the day following the operation. The aldosterone excretion was 3.2ug/24 hours pre-operatively and therefore lay within the normal range, but rose tenfold on the first and second post-operative days. This estimation was confirmed by duplicate analysis with different dosage levels per rat - the stippled areas in the figure representing the difference between the two estimations. On the third, fourth, and fifth post-operative days the aldosterone excretion had returned to a normal level and remained so on the sixth, seventh, and eighth post-operative days, by which time the patient was receiving 75 mg. of cortisone acetate per day in preparation for the second stage of the adrenalectomy. On this evidence it was concluded that the method used was sensitive enough to detect the increase in aldosterone excretion expected after a major operation in a patient with at least one intact adrenal gland, and could therefore be used to ascertain whether or not there was a comparable increase in

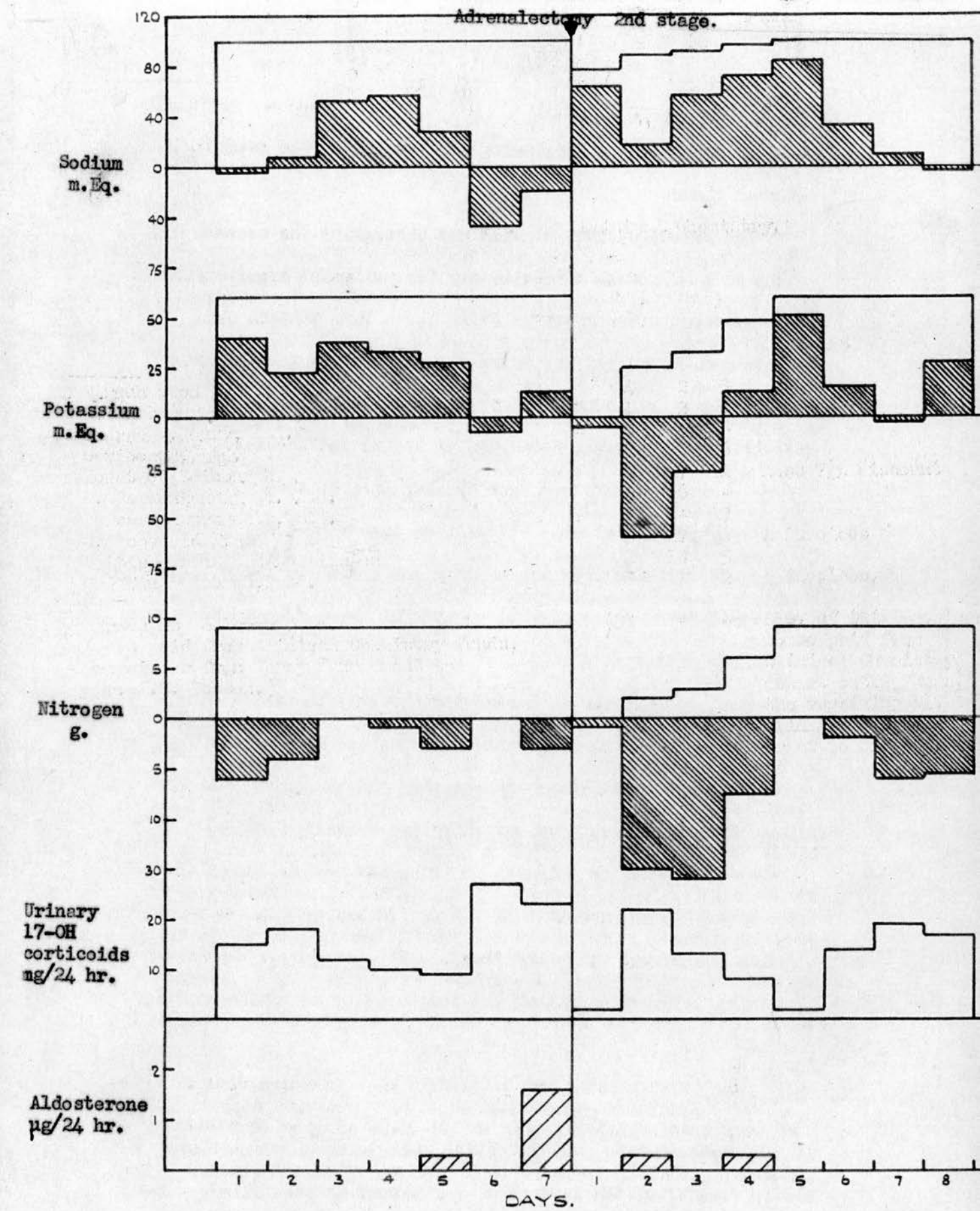


FIG. 2. D. McK. Sodium, potassium and nitrogen balances and 17-OH corticostero and aldosterone excretion before and after the 2nd stage of a bilatera adrenalectomy. Cortisone acetate 200 mg./day i.m. over period of observation.

aldosterone excretion following the removal of the second adrenal gland.

The second patient studied was undergoing the second stage of a bilateral adrenalectomy for malignant hypertension and the results are shown in Figs. 2. This patient was maintained on 200 mg. cortisone acetate per day given intramuscularly at six-hourly intervals. Despite this large dose of cortisone the usual retention of sodium and increased excretion of potassium and nitrogen were clearly seen in this patient following the operation. It will be noted, however, that there was definitely no increase in the amount of 17-OH corticosteroids excreted showing that no gross change had occurred, either as a result of increased metabolism of the exogenous steroid, or as a result of greatly increased output of hormones from the remaining adrenal gland in the brief period between the first skin incision and the removal of the gland from the body. On the third pre-operative day no aldosterone could be detected in the urine but on the immediate pre-operative day an excretion of 1.6 ug./24 hours, i.e. an amount within the normal range was found. No aldosterone whatsoever could be detected on either the second or fourth post-operative days.

The third patient was maintained on a constant dose of 100mg. of cortisone acetate per day whilst undergoing an ovariectomy eleven days after the removal of her second adrenal gland. The

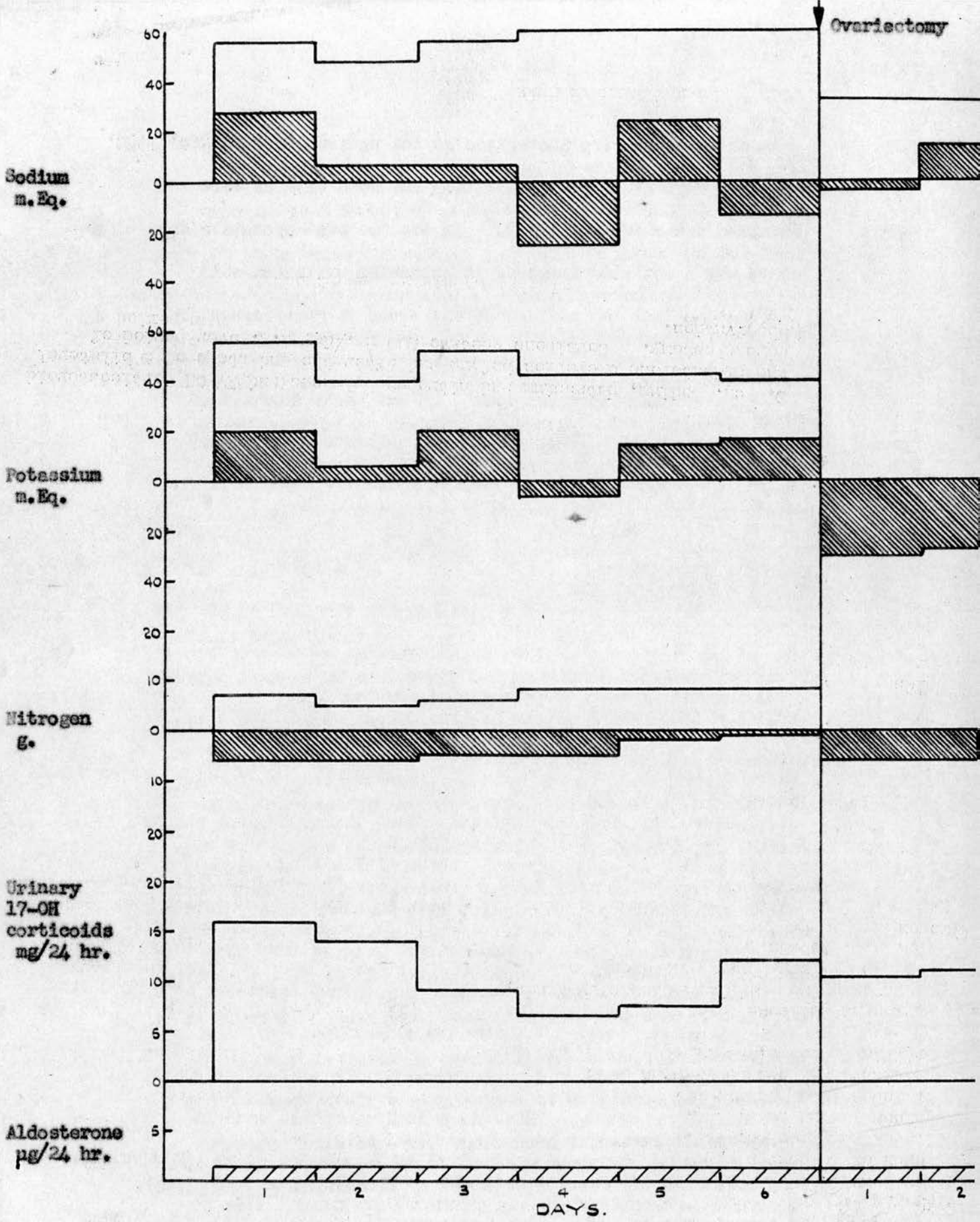


FIG. 3. F.R. Sodium, potassium and nitrogen balances and 17-OH corticoster and aldosterone excretion before and after ovariectomy following previous total adrenalectomy. Cortisone acetate 100 mg/day i.m. over period of observation.

data on this case are incomplete as the patient survived for only two days after the ovariectomy but such data as were obtained are shown in Fig. 3. In the two post-operative days there was a definite increase in potassium excretion with indications that the periods of increased nitrogen excretion and sodium retention had started. There was no increase in 17-OH corticosteroid excretion. As was to be expected no aldosterone could be detected in the urine of this patient on any of the six pre- and two post-operative days.

2. DISCUSSION

The choice of cases for settling the question of whether or not aldosterone excretion could continue or even be raised following the removal of the second adrenal gland, was rather unfortunate in that two of the patients died before the observations could be completed. However, several facts emerge from the results. Firstly, that the method for the determination of aldosterone in urine although laborious and crude was capable of detecting a rise in aldosterone excretion following a first-stage adrenalectomy. It is true that this one case may not be representative, may infact represent an exaggerated response. However the aldosterone excretion following the operation is approximately 300 percent of the pre-operative amount, a percentage increase which accords very well with the results obtained by Zimmermann et al. (1956) in seven patients undergoing abdominal surgery. Also the

urinary output of total steroids following any major operation not involving removal of both adrenal glands, usually shows a three- or four-fold increase and it would seem reasonable to expect that the excretion of aldosterone would show a comparable increase, as indeed it did in this case. It must be emphasized that this increase in aldosterone excretion occurred in conjunction with what must be regarded as an essentially normal metabolic response to trauma in respect to sodium, potassium, nitrogen, and total steroid excretion. In the absence of evidence to the contrary it can therefore be assumed that this rise in aldosterone excretion can be taken as representing yet another facet of the normal response.

Secondly, assuming the method for the determination of aldosterone to be adequate, the fact emerges that no aldosterone was detectable at any time following the removal of the second adrenal gland in any patient. It may be that some people excrete other substances antagonistic to aldosterone which render it inactive in any biological assay. However, the absence of any ultra-violet-absorbing spot in the aldosterone position on any of the paper chromatograms of the urines which gave negative results in the subsequent bioassay, would seem to make this possibility rather remote. Additional evidence on this point was supplied by the further four cases described in the next section in whom aldosterone determinations were also carried out before and after second-stage adrenalectomies.

All of these four cases, as will be seen, showed normal values for aldosterone excretion in the pre-operative period falling to very low or undetectable levels in the immediate post-operative period. It may be mentioned that in these later cases the aldosterone excretion was also measured by the physico-chemical method of Nowaczynski et al. (1956) and essentially the same results were obtained as by the biological method, namely normal values in the control pre-operative period and undetectable or trace values in the post-operative period.

It is noteworthy that the absence of aldosterone excretion following the removal of the adrenal glands in Figs. 2. and 3. is coupled with what in one case is, and in the other case promises to be, a normal metabolic response to operation including marked electrolyte changes. It is now established without a doubt that aldosterone is the main electrolyte-regulating hormone produced by the adrenal cortex under normal conditions. It has been postulated that an increase in aldosterone excretion could occur even after the removal of the second adrenal gland, either from some extra-adrenal source or from the liberation of aldosterone from some inert combination in the tissues or peripheral blood. This possibility would seem to be definitely ruled out by the results presented here. The possibility remains that some minute increase in the level of aldosterone circulating in the blood could occur for a short time before the body's supply of aldosterone became exhausted - perhaps as a result of an increase in aldosterone secretion by the remaining

adrenal gland in the brief period between the first incision and the actual removal of the gland - and that this increase could cause the subsequent metabolic events. It is, however, difficult to visualise the existence of such an extremely delicate regulating mechanism and it is even more difficult to imagine the existence of a mechanism which is not only so delicate but whose effects extend over such a long period after the primary stimulus has been removed. The possibility is obscure but a firm answer in the negative awaits the emergence of a method sensitive enough to measure the amounts of aldosterone present in reasonable volumes of blood, the amount of aldosterone present in human systemic blood being estimated at 0.08 - 0.10 ug./l. (Simpson and Tain, 1953) on the basis of values obtained in several litres of pooled human blood.

It appears necessary to look elsewhere for the primary stimulus to the chain of metabolic events which follow surgical procedures. Might not an alteration in the blood level of free adrenal steroids due to some alteration in the metabolism of the administered hormones, or to an increase in the output of endogenous hormone at the time of the start of the operation, provide such a stimulus as suggested by Steenburg and Ganong in 1955? The results recorded in the next section represent an attempt to answer this question.

3. PLASMA LEVELS OF FREE 17-OH CORTICOSTEROIDS FOLLOWING ADRENALECTOMY

In all of the previous studies on the metabolic response to adrenalectomy the patients were maintained over the period of the operation either on a fixed amount of cortisone per 24 hours given intramuscularly in divided doses, or on an intravenous infusion of hydrocortisone lasting for several hours and starting shortly before the commencement of the operation. Neither of these procedures was considered to be satisfactory from the point of view of examining the blood hormone levels in any patient. Very little is known of the rate at which intramuscular cortisone is utilised and it would be expected that it would be very difficult if not immpossible to maintain blood adrenocortical hormones at anything like constant levels over the 24 hours by this method. To give hydrocortisone intravenously for several hours covering the period of the operation is obviously of little use as it does not allow time for the blood levels to become stabilised, and in the time interval before the removal of the second adrenal gland the secretions of the gland are contributing an unknown quantity to the blood levels, this contribution ceasing abruptly at the time of the operation. It was decided therefore, to attempt to maintain the patients on a constant dose of intravenous hydrocortisone administered at a steady rate throughout the 24 hours, the administration starting several days prior to the operation with a view to

suppressing endogenous secretion and achieving steady blood levels; the infusion was to continue for several days after the operation, to allow the full results of the trauma to develop under constant conditions. This was achieved by dissolving the required 24 hour dose of hydrocortisone in 1800 ml. of 6% dextrose which was then administered to the patient by a catheter inserted into the superior vena cava. By this means it was found possible to maintain the patients on a constant dose of hydrocortisone for a week or more, the blood steroid levels, after the drip had been running for about 24 hours, remaining reasonably constant. It was thought that this method of steroid administration represented as close an approach to the physiological mode of output of adrenal steroids (in the absence of any kind of "stress") as is possible in the present state of our knowledge.

The group studied under this regime comprised four female patients undergoing the second stage of a bilateral adrenalectomy, the operation being undertaken for carcinoma of the breast in two cases, for malignant hypertension in one case, and for Cushing's syndrome unresponsive to cortisone treatment in the fourth case. The results in the case of the patient suffering from Cushing's syndrome turned out to be quite anomalous and will therefore be considered separately. The results obtained in the other three cases are shown in Figs. 4-7.

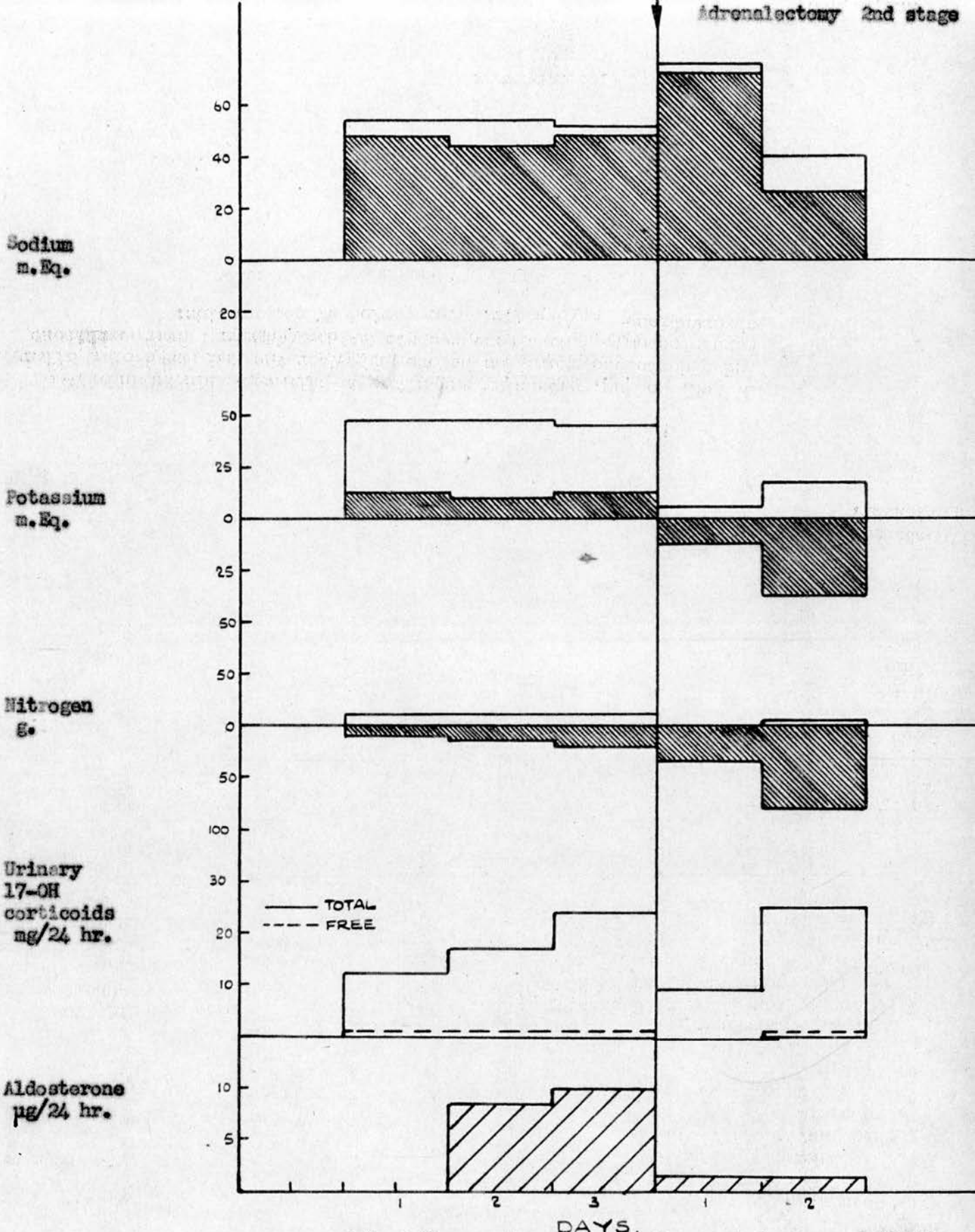


FIG. 5. I.N. Sodium, potassium and nitrogen balances and 17-OH corticosteroid and aldosterone excretion before and after 2nd stage of a bilateral adrenalectomy. Hydrocortisone 150 mg/day i.v. over period of observation.

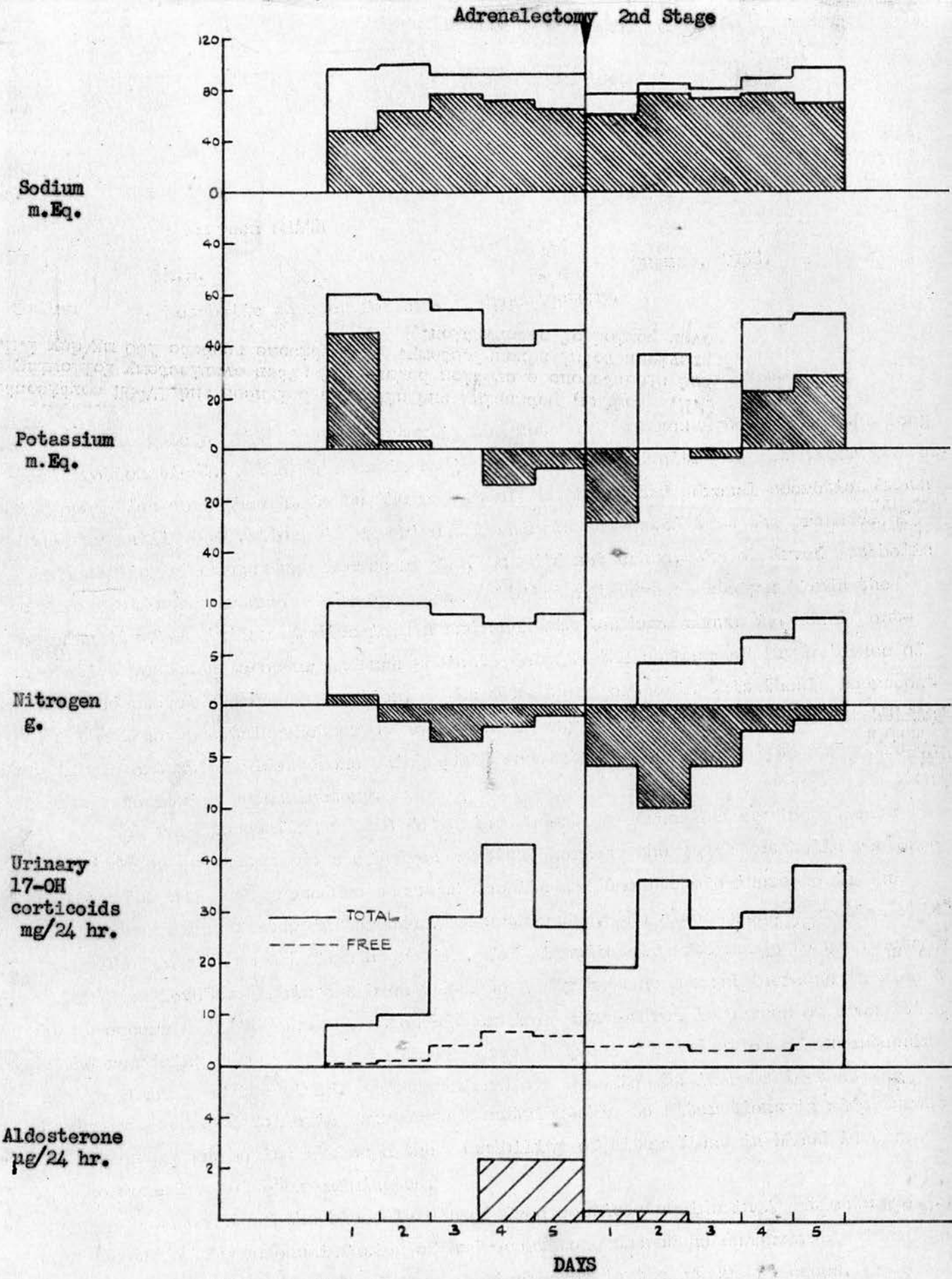


FIG. 4. H.L. Sodium, potassium and nitrogen balances and 17-OH corticosteroid and aldosterone excretion before and after 2nd stage of a bilateral adrenalectomy. Hydrocortisone 150 mg/day i.v. over period of observation.

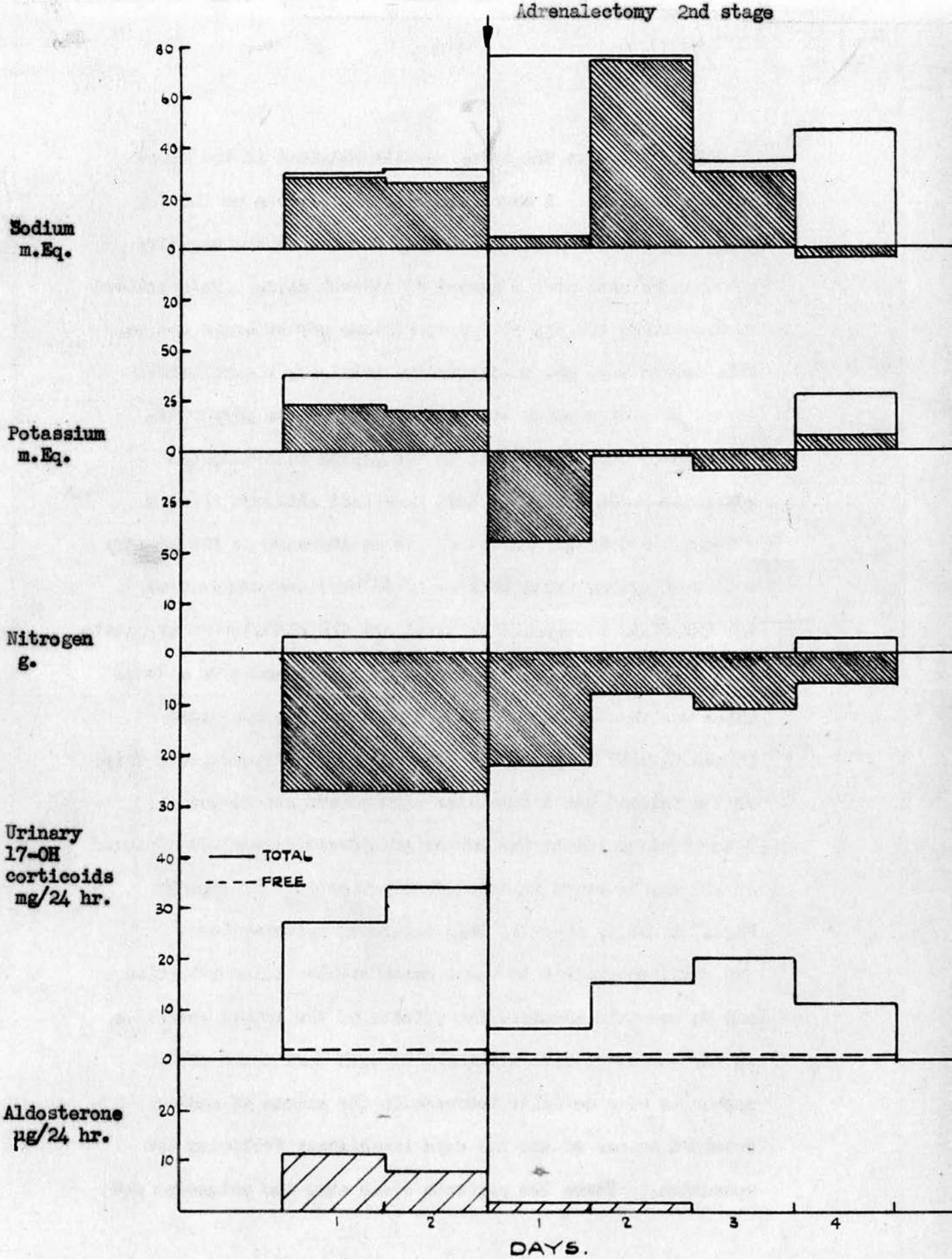


FIG. 6. M.K. Sodium, potassium and nitrogen balances and 17-OH corticosteroid and aldosterone excretion before and after the 2nd stage of a bilateral adrenalectomy. Hydrocortisone 150 mg/day i.v. over period of observation.

Fig. 4. shows the urine results obtained in the first patient studied. A negative potassium balance on the day following the operation is clearly seen as is the negative nitrogen balance over a period of several days. This patient was receiving 200 mg. of Hydrocortisone per 24 hours and on this dosage was, not unexpectedly, retaining a considerable amount of sodium prior to the operation. The proportion of ingested sodium retained in the period following the operation would appear to have increased although this is a debatable point. There was not an increase in the urinary output of either total or free 17-OH corticosteroids from the immediate pre-operative level and the aldosterone excretion fell from a pre-operative level of 2 µg./24 hours to a level which was undetectable. Five days after the operation it was thought advisable to take down the hydrocortisone drip as the patient had accumulated some oedema and it was determined to reduce the amount of hydrocortisone administered to 150 mg./24 hours in subsequent patients. As seen in Figs. 4. and 5, however, this dosage of hydrocortisone was still sufficient to cause considerable sodium retention per se and this obscured the effects of the actual operation on the sodium balance althought in each case there would appear to be a definite increase in the amount of sodium retained on one of the two days immediately following the operation. These two patients again show the potassium and

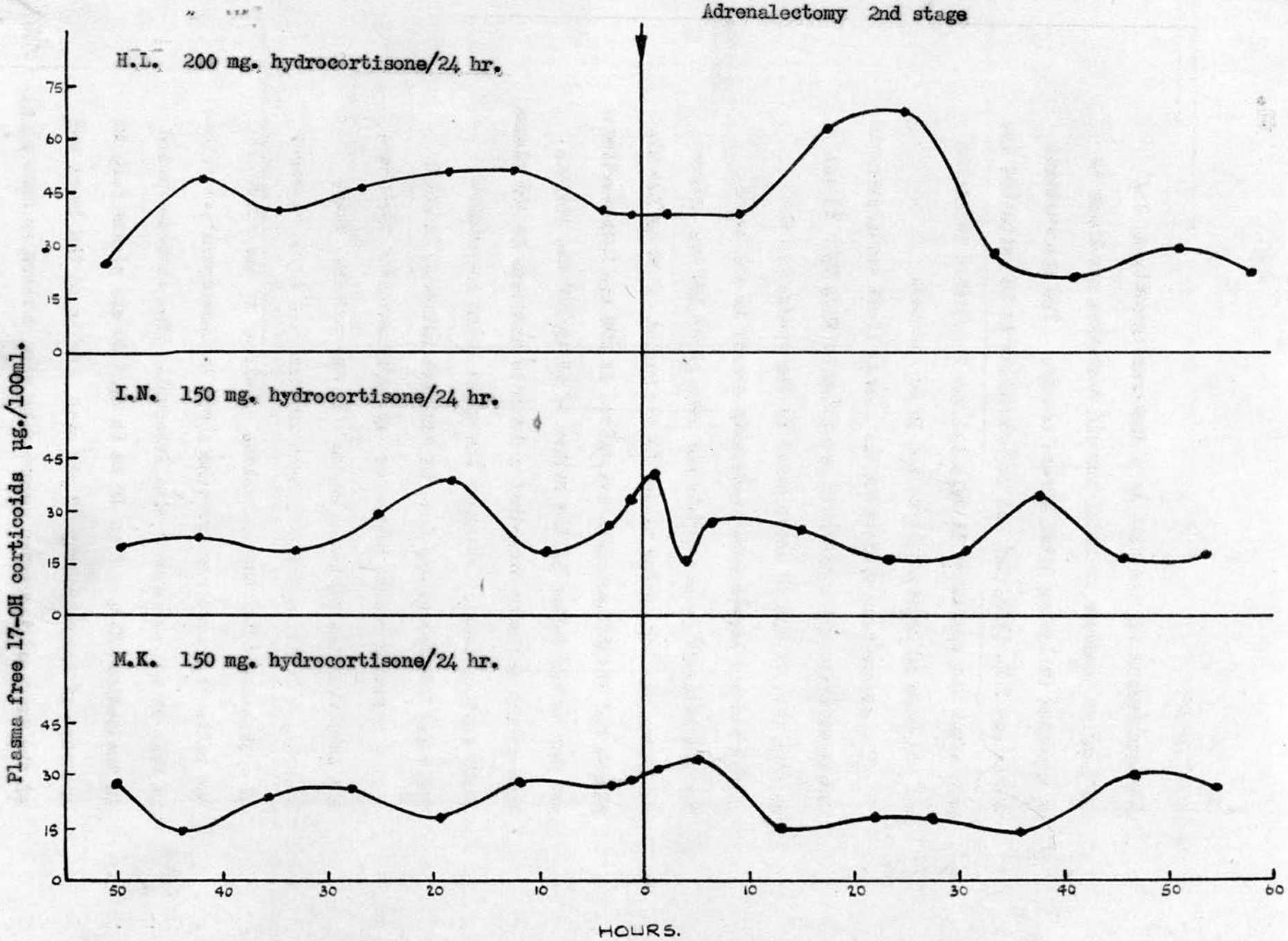


Fig. 7. Plasma steroid levels before and after 2nd stage adrenalectomy in patients receiving constant i.v. hydrocortisone.

nitrogen responses, the fall in aldosterone excretion, and the lack of increase in total steroid excretion now known to be expected following total adrenalectomy. The observations on patient I.N. (Fig. 5.) had unfortunately to be curtailed two days after the operation as this patient complained of angina and the dose of hydrocortisone had to be reduced.

The concomitant changes in the plasma 17-OH corticosteroid levels of these three patients are shown in Fig. 7. It can be seen that in the 48 hours prior to the operations the plasma steroid levels were reasonably steady in all cases, the two patients I.N. and M.K. who were given 150 mg. hydrocortisone per day having values in the region of 30 µg/100 ml. plasma and the patient H.L. maintained on 200 mg. hydrocortisone per day having values in the region of 45 µg/100 ml. plasma. None of the patients exhibited a definite increase in the plasma 17-OH corticosteroid levels in the eight hours immediately following the operation, i.e. in the time interval in which a rise normally occurs following any major surgical procedure not involving the adrenal glands. In one patient, H.L., however, a definite though small increase was found to occur 10 - 30 hours after the operation. Neither of the other two patients showed any increase above the pre-operative values in this or any subsequent time interval. The increase noted in the patient H.L. - from 40 to 65 µg./100 ml. plasma i.e. an increase of approximately 60 per cent - falls at the lower end of the range of increases reported by such workers as Swan et al. (1957)

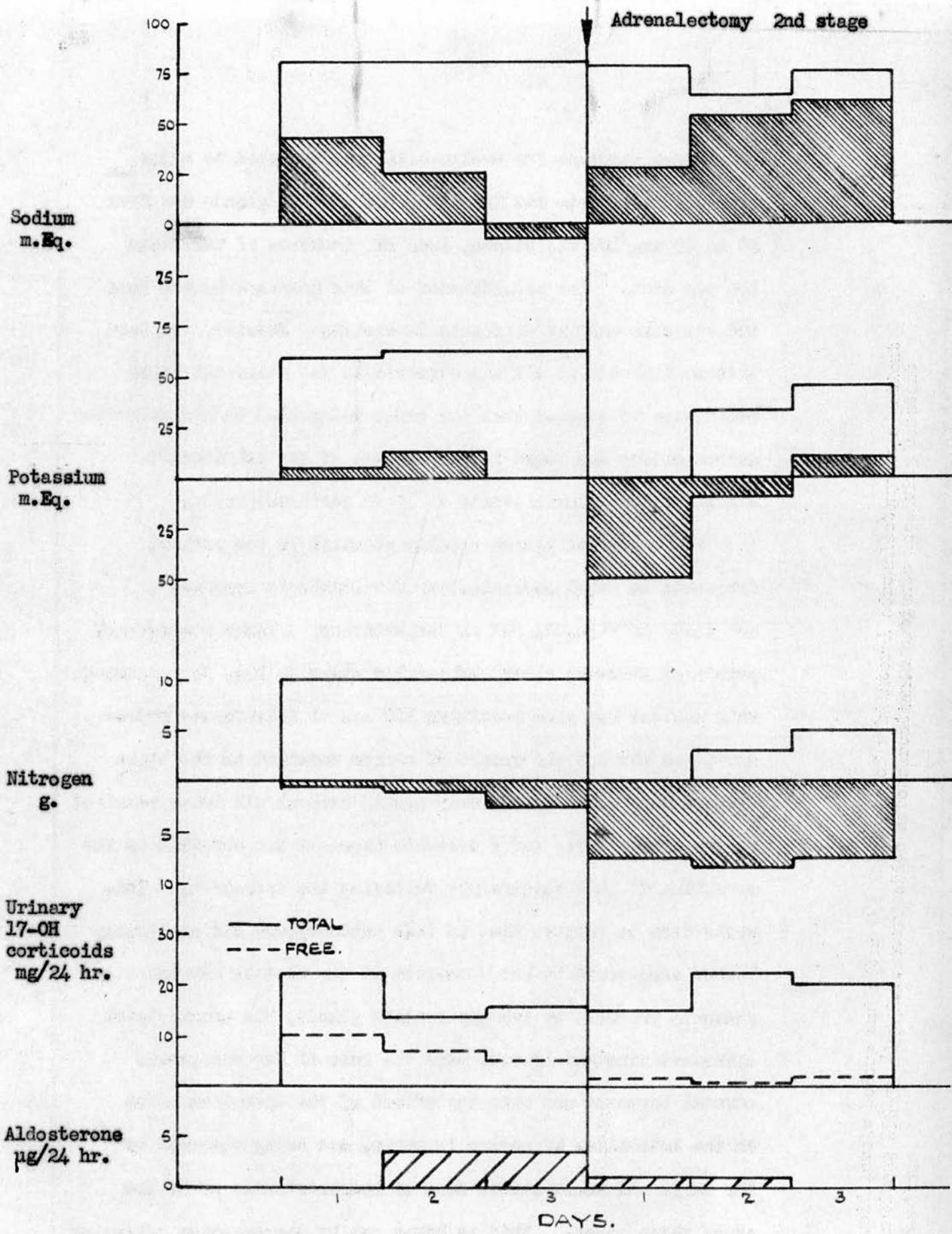
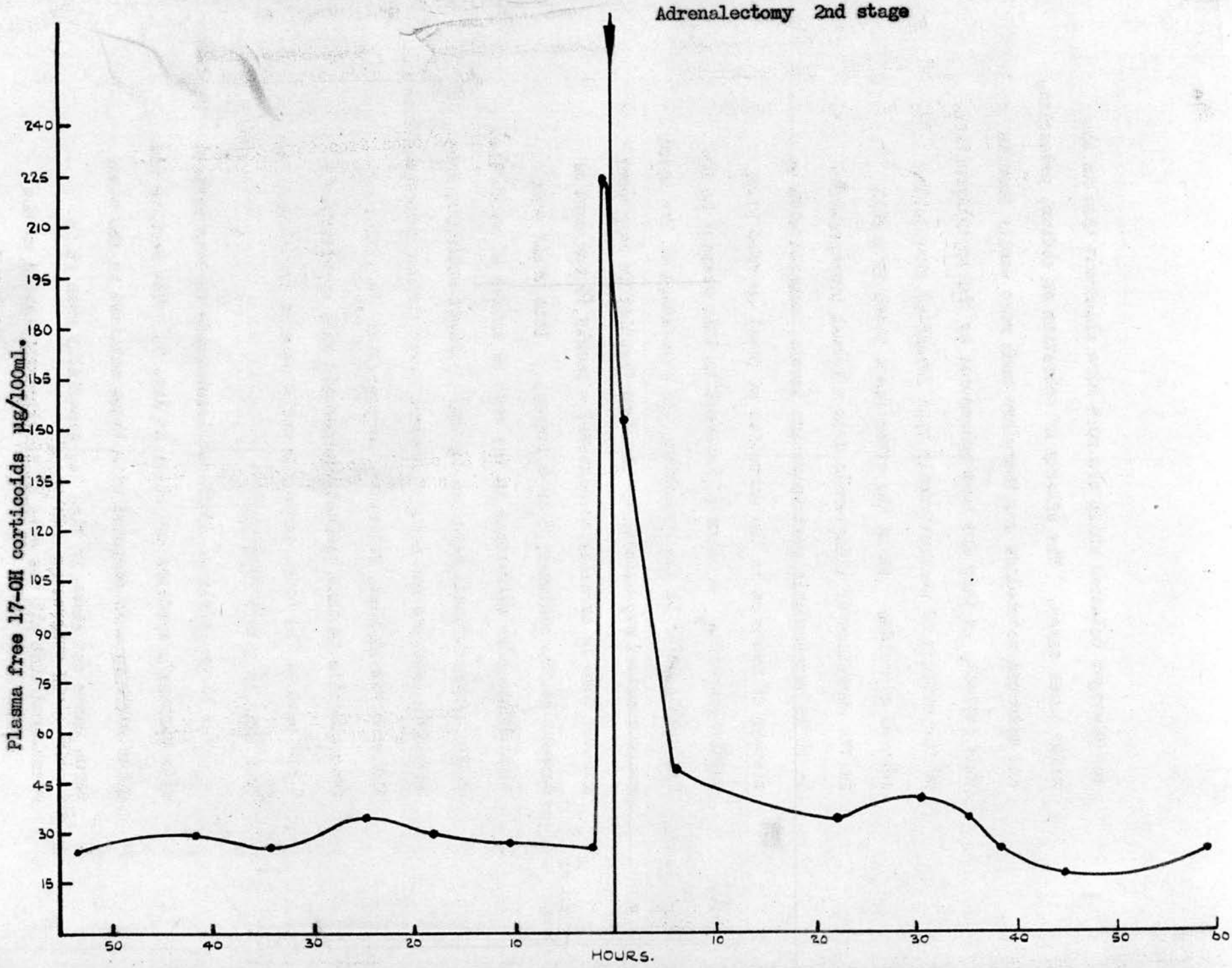


FIG. 8. J.D. Sodium, potassium and nitrogen balances and 17-OH corticosteroid and aldosterone excretion before and after the 2nd stage of a bilateral adrenalectomy. Hydrocortisone 150 mg./day i.v. over period of observation.

whose mean increase for twelve patients subjected to major surgical procedures not involving the adrenal glands was from 18 to 45 µg./100 ml. plasma, i.e. an increase of more than 100 per cent. The significance of this increase in one case and one case only is difficult to assess. However, the fact that no increase at all was observed in the other two cases would seem to suggest that the usual metabolic changes following adrenalectomy can occur in the absence of any significant changes in the plasma levels of 17-OH corticosteroids.

The urine and plasma results obtained in the patient subjected to total adrenalectomy for Cushing's syndrome are shown in Figs. 8. and 9. respectively. There are several points of interest about the results shown in Fig. 8. Although this patient was also receiving 150 mg. of intravenous hydrocortisone per day the amount of sodium retained in the three control days was comparatively small, none at all being retained on one of the days, and a definite increase was observed in the retention of this electrolyte following the operation. This would seem to suggest that in this patient, who had presumably become accustomed to large amounts of circulating adrenal steroids produced by her hyperactive glands, the administered hydrocortisone merely took over the role of her endogenous adrenal hormones and thus the effect of the operation alone on the metabolism of sodium is shown, not being obscured by the large and unaccustomed dose of hydrocortisone as in the other three cases. This is borne out by the negative potassium


Adrenalectomy 2nd stage
Plasma free 17-OH corticoids µg/100ml.
240
225
210
195
180
165
150
135
120
105
90
75
60
45
30
15
50
40
30
20
10
10
20
30
40
50
60
HOURS.

and nitrogen balances which are much more clear-cut than in the other three cases. The effects of operation on sodium, potassium, and nitrogen metabolism are therefore much more easily seen in this patient, as they are here unhampered by the superimposition of the effects of unaccustomedly high levels of circulating adrenal steroids. As in the other cases there is a fall in the excretion of aldosterone from a normal pre-operative level to undetectable post-operative levels, coupled with an absence of increase in the excretion of total or free 17-OH corticosteroids. A point of interest in this respect is the fact that, prior to the operation, the percentage of the total steroid output represented by the free fraction is very much greater than is normally encountered - nearly 50 per cent as opposed to the customary 5 or 6 percent. This would seem to indicate some difference in the rate or manner of metabolism of the adrenocortical hormones in this patient suffering from Cushing's syndrome and would therefore merit further investigation. The observation that, following the operation the output of free steroids in this patient represented only approximately 5 per cent of the total excretion would seem to indicate that this is a true finding.

The blood levels of 17-OH corticosteroids in this patient with Cushing's syndrome are shown in Fig. 9. The results are quite striking when compared with those obtained in the other three cases and shown in Fig. 7., especially when it is remembered that all the sets of results are plotted on the

same scale. The enormous increase in blood steroid levels observed occurred immediately following the induction of anaesthesia and then quickly fell to just above the pre-operative levels within six hours, a more gradual fall continuing for a further twenty-four hours. This increase clearly represented the exaggerated response of a hyperactive Cushinoid adrenal gland to the stress of anaesthesia, and indeed such high levels of plasma 17-OH corticosteroids have previously only been reported in patients with Cushing's disease in reponse to ACTH administration. The fact that this great increase is in fact due to an exaggerated response of the remaining gland in the interval between the induction of anaesthesia and the removal of the gland from the body, would appear to be borne out by the rapidity with which the plasma levels fell to near normal values - within six hours i.e. well within the period during which the response to the operation itself would be expected to be still manifested. This case, although contributing little to the thorny problem of whether or not an increase in the blood levels of 17-OH corticosteroids could be the initiating factor in the metabolic response to surgery, proved to be of intrinsic interest and has therefore been included in this series of observations.

4. DISCUSSION

It is indeed unfortunate, although not entirely unexpected, that the dose of intravenous hydrocortisone given to these patients was such as to cause a degree of sodium retention which

largely obscured the effects of the operation on sodium metabolism. Comparable doses of intramuscular cortisone acetate do not cause sodium retention to any marked degree and this may be taken as yet another example of the fact thtat substances administered intravenously are utilised much more readily than substances administered by any other route. The obvious way to overcome this difficulty would be to start patients undergoing adrenalectomy on intravenous hydrocortisone very much earlier, so that the patients could become accustomed to high levels of circulating adrenal hormones and the "escape mechanism", so often seen during the prolonged administration of adrenal steroids, could come into play, the sodium metabolism eventually reaching a state of equilibrium under this high dosage level. As well as being very tedious and possibly requiring weeks of continuous intravenous hydrocortisone infusion this treatment would be fraught with obvious danger to the patient in the form of accumulation of large amounts of oedema fluid and the results would hardly be worth the trouble and risk taken. Nature has, however, done this experiment for us in the case of the patient suffering from Cushing's syndrome who was presumably accustomed to high circulating adrenal steroid levels and in whom the intravenous administration of 150 mg. hydrocortisone per 24 hours did not cause such marked sodium retention that the increased retention following operation could not be clearly seen. The post-operative sodium retention, when coupled with the usual negative nitrogen and potassium balances seen post-operatively in the other

three cases, would suggest that the post-operative sodium response had occurred although it had been obscured.

It is now well established that no increase in the output of total or free urinary 17-OH corticosteroids is to be expected following adrenalectomy and this was confirmed in all four cases. It must be remembered, however, that whereas after injection of isotopically labelled steroids over 90 per cent of the radioactivity is recoverable in urine, less than 40 per cent of the administered radioactivity is found in the urinary steroids, whatever method is used to assess the steroid content. This points to the fact that some major metabolite or metabolites of the adrenocortical hormones are not measured by any of the existing methods and so a small but real increase in the output of adrenal hormones might well escape detection. The measurement of the urinary output of steroid hormones can tell us little if anything of the fluctuations in the blood levels of these hormones. This is well demonstrated by the very large though transient increase in the blood levels of free 17-OH corticosteroids in patient J.D. (Fig. 9.) following operation, an increase which is not reflected by any rise in this patient's urinary output of 17-OH corticosteroids. In all of the patients except J.D. the free urinary 17-OH corticosteroids reflected a very small, unvarying, and presumably unimportant, percentage of the total 17-OH corticosteroid output. The question may indeed be raised as to whether this small percentage of free urinary steroids may not be an artefact produced by the action of other urinary constituents

on the steroid conjugates rather than representing a portion of the blood free steroids cleared by the kidneys as such. This question remains to be explored as does the interesting observation that in the patient with Cushing's syndrome, J.D. (Fig. 8.) before adrenalectomy nearly 50 per cent of the excreted steroids were in the free form, this percentage dropping to the usual 5 or 6 per cent immediately following the removal of the second adrenal gland.

The fall in the aldosterone excretion following the removal of the second adrenal gland seen in all four cases seems to be unequivocal, especially when it is remembered that the aldosterone values were confirmed independently using a second, physico-chemical, method, and rules out the possibility that continued renal aldosterone excretion could be responsible for the sodium conservation which continues for several days after removal of both glands, Aldosterone may still, of course, be present in small quantities in the blood but it is difficult to see how small, and presumably diminishing, quantities of circulating aldosterone could bring about an event such as the increased sodium conservation, lasting for a number of days.

In the absence of continued aldosterone excretion following adrenalectomy it is almost essential to assume either that (a) transient changes in blood corticoid levels occur and are sufficient to "trigger off" the metabolic events that follow in the next few days or (b) that the adrenal cortex and its steroid

products bear no relation to post-traumatic metabolic events other than the "permissive" one described by Ingle. The results reported in the preceding chapter would seem to indicate that (a) does not hold. It is true that in patient H.L. (Fig. 7.) an increase in the free blood 17-OH corticosteroids was found to occur 24 hours after the operation. This increase was not, however, very convincing being neither as early nor as large as the increases usually reported following comparable operations not involving the adrenal glands. It is also true that the patient, J.D., with Cushing's syndrome (Fig. 9.) showed a striking increase in blood free steroid levels. This rise, however, was very atypical, the highest value being obtained in the period between the induction of anaesthesia and the start of the operation and the levels returning to near normal with in 8 hours of the end of the operation at a time when the usual post-operative rise is just approaching its maximum. That this represents the excessive response of an overactive adrenal gland to the stress of anaesthesia would seem to be borne out by the fact that the only comparable rises have been obtained in subjects with Cushing's syndrome following injection of ACTH. Balanced against these two atypical blood steroid increases following adrenalectomy we have the results in the other two patients, I.N. and M.K. (Fig. 7.), showing no increase in free steroid levels whatsoever following adrenalectomy. When taken in conjunction with the small but definite number of cases reported in the literature and already mentioned, as having shown no rise in blood steroid levels following a variety of major

surgical procedures not involving the adrenal glands, the conclusion seems practically inescapable that the metabolic events which normally follow surgical trauma can occur in the absence of any measurable rise in circulating steroids and therefore that no part of the metabolic response to surgery can be initiated solely by an increase in the secretion of adrenocortical hormones. The only apparently conflicting evidence comes from the work of Steenburg and Ganong (1955) on adrenalectomized dogs subjected to various kinds of trauma including laparotomy. However, their results must be treated with a certain amount of reserve as their animals were only maintained for a very short time on intravenous hydrocortisone and no control values were obtained to ensure that the blood steroid levels had reached equilibrium. The post-operative increases in the blood free steroid levels obtained by these workers in dogs, as well as the increase seen in the patient H.L., may well be attributable solely to a delay in conjugation of the free steroids by the liver. It is now well established that liver damage may play a part in contributing to the increased blood steroid levels following major operations but the conflicting results obtained by different workers who have tried to correlate the magnitude of the post-operative steroid increases with the degree of liver damage would appear to suggest that the part played by the liver is of varying importance in different patients. The suggestion that delayed liver conjugation may play no part at all in

producing the increased post-operative free steroid levels in some patients, is borne out by the results of two groups of workers (Sandberg et al., 1954; Elman et al., 1955) who infused hydrocortisone into several patients on control and post-operative days and found no difference in the rate of disappearance of the infused steroid or appearance of glucuronide conjugates on the two days, both sets of workers concluding that there is no difference in the metabolism of exogenous, and, by analogy, endogenous steroids immediately following operation.

It is true that no measurements of the blood levels of conjugated 17-OH corticosteroids were made in the patients subjected to investigation but it is difficult to conceive how any changes in the blood levels of these conjugates - for which no biological activity has yet been claimed - could bring about the metabolic response. It may be argued that the blood levels of free steroids may indeed be constant but that this constancy may be achieved by the rates of steroid secretion and of conjugation varying together, this constant level of free steroid therefore representing the fixed resultant of an infinite variety of rates of metabolism. But under the conditions of the experiments presented here when hydrocortisone was infused at a steady rate over the 24 hours, no great variation in the rate of appearance of free steroid in the blood seems possible unless it is postulated that much of the infused steroid is rapidly stored, perhaps in the cells, in some inert form and

is only released when the blood levels of free steroid fall. An analogy could be drawn here with potassium metabolism, it now being well known that the serum potassium level is maintained at the expense of intracellular potassium. But what then could be the initial cause of the postulated fall in the blood free steroid levels? It is exceeding difficult to see what cause there could be for an increase in the rate of conjugation as the primary factor, quite apart from the fact that a large number of workers have shown the existence of decreased conjugation following surgery. Increased tissue utilisation of free steroid would be understandable but no evidence for its existence has ever been put forward. Indeed, until the primary sites of action of the adrenal steroid hormones have been uncovered, it is difficult to know where to look for such evidence. The great weakness in the theory that either increased conjugation or increased tissue utilisation could lower the levels of circulating free hormone which is then replenished from other (e.g. intracellular, sources) is that no decrease has been observed, even immediately after the most severe trauma, in the level of free blood steroids. Indeed all the available evidence points to the contrary - that there is in fact no homeostatic mechanism controlling blood hormone levels. Blood levels of both free and conjugated steroids can be quite markedly elevated and for a considerable time by infusions of ACTH and hydrocortisone. And definite confirmation of the lack of a homeostatic mechanism would appear to come from the observation

(Hellman et al., 1954) that indentical doses of labelled hydrocortisone given to normal persons and to an adrenalectomized patient off maintenance steroid therapy were metabolised in a seemingly identical manner, thus showing that the metabolism of the administered steroid was independent of the body's need. It must also be noted that this adrenalectomized patient showed the characteristic renal changes of adrenal insufficiency, including reduced urine flow, demonstrating that the renal factors are of no great importance in regulating steroid metabolism.

It may be mentioned in passing, that although observations on the patients reported in this thesis were carried out under conditions which were kept as nearly constant as possible by means of a continuous intravenous infusion of hydrocortisone, it was realised that no fixed dose of any one adrenal hormone represents anything approaching the physiological state. It is well recognized that the effects of any one adrenocortical hormone are modified by the concurrent effects of the other adrenal hormones and it may be that the adrenal gland can alter the secretion of the various hormones independently thus tailoring its effluent to the needs of the moment, a state of affairs which would be very difficult, if not impossible, to reproduce artificially. Also it has been shown in dogs (Hume and Nelson, 1954) that the adrenal output appears to be intermittent, a fact borne out by the marked diurnal variation in both blood and urine 17-OH corticosteroids found in man. Until, however, a lot more is known with certainty about the stimulus to adrenocortical secretion in man it will

be difficult, if not impossible, to approach more closely to the physiological state using exogenous steroids alone.

It was long thought that a lowering of the blood levels following trauma caused the anterior pituitary to secrete more ACTH which in turn stimulated the adrenal glands to secrete more hormones, and that conversely a raising of the blood levels from any cause produced a prompt reduction in the secretion of ACTH with a consequently reduced adrenal outflow. Alas, it is now recognized that the mechanism is not nearly so straightforward! Trauma of any kind causes a release of ACTH within as short a time interval as 10 seconds but the depression of ACTH produced by the prolonged administration of cortisone or allied steroids may take days or even weeks to be fully effective. Prolonged stress produces both increased amounts of adrenocortical hormones and adrenal hypertrophy showing that there is prolonged ACTH secretion in spite of high blood steroid levels. No lowering of the blood steroid levels has ever been demonstrated following trauma, likewise no increased tissue demand for the steroid hormones of the adrenal cortex can be shown to exist. The work of Hume and others on dogs has shown that the hypothalamus is in some manner implicated in the increased blood steroid levels found after trauma. Taking all these points into account it has become increasingly apparent with the passage of time that following stress of any kind and the acute stress of operation in particular, factors other than ACTH are in part responsible for the increased adrenal steroid output. How else is it

possible to explain the increased output of 17-OH corticosteroids found following hypophysectomy (Robson et al., 1956), or the greater than normal increase in blood steroid levels obtained in response to ACTH injection in the immediate post-operative period? This other pathway leading to adrenal stimulation (which may well include neural factors) would surely repay further investigation. It might even provide the key to the metabolic response to surgery. The primary stimulus to the various metabolic events which follow surgery is obscure. The primary stimulus by which increased secretion of the adrenal cortex is brought about is still not fully understood. It is known that under normal circumstances increased adrenal secretion and the metabolic events consequent on surgery occur simultaneously or very nearly so, although by a judicious choice of circumstances including the cases cited in the last chapter, the two chains of events can be separated. Is it not possible that the stimulus to increased adrenal secretion and the stimulus to the metabolic events following surgery may prove to have one and the same origin? What does now appear to be reasonably certain is that the one does not cause the other - the stimulus to the metabolic consequences of surgery is not to be found among the hormones of the adrenal cortex.

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The results presented in this thesis would appear to provide additional evidence that the simple theory that the metabolic events following surgery are initiated by an increased secretion or an alteration in metabolism of one or all of the adrenocortical hormones, is no longe tenable. The patients were studied under conditions which precluded a prolonged increase in adrenal gland secretion at the time of the operation and yet all of them exhibited essentially normal metabolic responses to the stress of surgery. Two of the patients as well as exhibiting no increased urinary steroid output showed no measurable alteration whatsoever in their blood steroid levels following adrenalectomy. It may be that the changes in blood levels by which the adrenocortical hormones exert their influence are microscopic rather than macroscopic and that the methods used are not sensitive enough to detect these changes. Experiments with hydrocortisone infused on operative and control days have shown no macroscopic difference in the mode of metabolism of the infused steroid following operation - similar experiments using steroids labelled with radioactive isotopes should be able to demonstrate the presence of absence of any microscopic difference. The existence of such a delicate regulating mechanism, however, is to be strongly doubted, especially when it is realised that the smallest effective increase in blood levels would have to be considerably smaller than the normal diurnal variation encountered in any one individual.

Supposing that small, transient, and as yet unmeasurable, alterations in adrenocortical hormone metabolism do occur following adrenalectomy, qualitatively but not quantitatively similar to the transient but measurable alterations which usually, but not always, occur following other major operations it is necessary to assume (a) that the transient changes in adrenocortical hormone metabolism are sufficient to "trigger off" the metabolic events occurring in the next few days or (b) that the hormones of the adrenal cortex bear no relation to post-traumatic metabolic events other than the "permissive" one describe by Ingle. The former theory is unattractive on the grounds that it has no analogue in other fields of endocrine activity in all of which a discrete stimulus produces a discrete and temporally limited effect. However, Kelly et al., (1954) showed that after giving a normal person a single intravenous dose of 100 mg. hydrocortisone sodium retention persisted for 24 hours whereas the blood levels of 17-OH corticosteroids returned to normal within 8 hours. This was one of the first examples reported of a prolonged response to a single stimulus. Further examples have come from the work of Barrter et al., (1956) and Wolff (1958) who showed that transient reductions in blood volume may be associated with a diminished excretion of sodium that lasts for three to four days and that aldosterone activity remains high for a similar length of time. However, the absence of aldosterone from the post-operative urine of the adrenalectomized patients studied here implies that this mechanism cannot be directly responsible in all circumstances.

Further evidence against the causal association of changes in blood corticosteroid concentrations and in sodium excretion is provided by the knowledge that a dose of pituitary ACTH which is sufficient to produce changes in blood levels of 17-OH corticosteroids of the order and duration seen after major surgical procedures (Steenburg et al., 1956) is associated with only a transient reduction in renal sodium excretion (Forsham et al., 1948). Therefore it would seem probable that the origin of the changes that are observed after operation must be sought not in alterations in adrenocortical activity but in some other physiological adjustments, the nature of which is as yet unknown.

Future research into the causes of the metabolic events which follow surgery would probably meet with most profit if undertaken with the clarification of the cause of the prolonged sodium retention as its main object. The alterations in the renal excretion of potassium and nitrogen would appear at present to bear a closer relationship to the extent of tissue injury and the size of the wound haematoma than to any other factor. The available evidence suggests that the magnitude of nitrogen loss is not determined by endocrine factors although the presence of adrenocortical hormone is permissive to its occurrence in the experimental animal and presumably also in man. The use of isotopic potassium and nitrogen in man would provide useful information as to the source of the increased urinary potassium and nitrogen observed following operation and would help to solve

the problem as to whether or not these increases were intrinsic features of the metabolic response. The prolonged sodium retention is, however, both the most consistent and the most puzzling feature of the metabolic response. Although correlating reasonably well with increased adrenocortical secretion when at least one adrenal is intact and functioning it also consistently occurs when there is no apparent possibility of variation in the concentration of adrenocortical hormones. It was long thought that increased secretion of aldosterone at the time of operation could account for the prolonged renal sodium conservation. However, the results reported in this thesis show that in the majority of cases no aldosterone is detectable in the urine following adrenalectomy although marked sodium retention is still evident. There may still, of course, be fluctuations in the amounts of aldosterone circulating in the blood although it is difficult to see how such fluctuations could produce the effect observed. Once again, the use of labelled aldosterone would appear to be the only practicable way of measuring the amounts of circulating aldosterone and thus settling this point. Two other possibilities exist; (a) that unmeasurable changes in renal blood flow, the result perhaps of a small post-operative reduction in blood volume, persist for several days after operation, a possibility that cannot be denied when it is recalled that all the physiological changes in sodium excretion can be accounted for by changes in glomerular

filtration rate too small to be measured (see Wesson, 1957, for review); (b) that the kidney has in some way a "memory" in its cells which results in the persistence of sodium conservation long after the removal of the initial stimulus - a similar delayed response system being implicit in the observations of Chalmers et al. (1952) on sodium excretion during reduction in glomerular filtration rate, in which it was demonstrated that sodium excretion was still diminishing when glomerular filtration rate had returned to normal. Whichever possibility proves to be correct it is evident that sodium metabolism is governed in some circumstances by some factor other than aldosterone. This is supported by the observations of Muller et al. (1958) that the diurnal variations in sodium and aldosterone output can be varied independently. It may well be that when we know what it is that governs sodium excretion when it is not under the influence of aldosterone, then, perhaps, we may have some inkling as to what it is that sets off that chain of events known as the metabolic response to surgery.

SUMMARY

SUMMARY

1) It has been confirmed that following second-stage adrenalectomy in patients maintained on a constant dose of adrenocortical hormone the usual metabolic response to any surgical procedure, consisting of urinary loss of potassium and nitrogen and a marked retention of sodium lasting for several days, occurs, despite no demonstrable increase in the urinary excretion of free or total 17-OH corticosteroids. This is regarded as proof that no gross changes in secretion of adrenocortical hormones had occurred during the removal of the second adrenal gland which could be considered as causing the other metabolic events.

2) A study of the output of aldosterone before and following second-stage adrenalectomy has shown that the pre-operative output of this hormone, which in all cases was within the normal range, fell immediately after operation to very low or, more usually, undetectable levels. This is taken to imply that no marked increase in aldosterone output occurred during the operation either from stimulation of the remaining adrenal gland in the brief period between the induction of the anaesthesia and the removal of the gland, or from extra-adrenal sources. It is therefore thought improbable that the electrolyte changes observed following surgery are due to an alteration in aldosterone production or metabolism, although alterations in blood levels of this hormone cannot entirely be ruled out for the present.

3) Measurement of the blood levels of free 17-OH corticosteroids in four patients undergoing adrenalectomy while maintained on a constant intravenous infusion of hydrocortisone has shown no demonstrable increase in two cases, an atypical and not very convincing increase in one case, and very marked increase typical of the exaggerated response of a hyperactive adrenal gland in the fourth case who was indeed a patient suffering from Cushing's syndrome. Although the sodium retention following the adrenalectomy was partially obscured in two of the patients because the dose of intravenous hydrocortisone was already causing sodium retention, these results are taken to imply that the metabolic response to surgery can take place in the absence of any measurable alteration in the metabolism of the adrenocortical hormones.

4) It is concluded that the primary stimulus for the metabolic events which follow surgical trauma must lie elsewhere than in the hormones of the adrenal cortex. It is suggested that until such time as the potassium and nitrogen responses have been shown by the use of radioactive isotopes to be intrinsic features of the metabolic response and not incidental by-products it is best to regard the sodium retention as the only fundamental aspect of the response. In this connection the view is tentatively put forward that the factor or factors which control sodium metabolism when it is not under the influence of aldosterone may well bear some connection to the factor or factors which initiate the metabolic response to surgery.

It is also suggested that the factors which stimulate the adrenal glands at operation and those which cause the metabolic events may have a common origin in some physiological adjustment the nature of which is as yet undetermined.

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THE RELATIONSHIP OF ALDOSTERONE EXCRETION TO THE METABOLIC RESPONSE TO ADRENALECTOMY

by

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INTRODUCTION

In a previous communication[1] we presented the results of balance studies of three patients undergoing 2nd stage adrenalectomy. They showed that increased urinary excretion of potassium and nitrogen and decreased urinary excretion of sodium and chloride occurred for several days in the immediate postoperative period. These changes, which constitute part of the metabolic response to surgery, were unaccompanied by an increase in the rate of urinary excretion of steroids such as is normally found after operations of comparable severity in patients with intact adrenal glands and after first stage adrenalectomy. Similar observations have been made by other workers[2,3], and the hypothesis that the alterations in nitrogen and electrolyte balance are wholly the result of an increased rate of secretion of adrenocorticosteroids[4-6] is no longer tenable.

With the discovery of aldosterone and its isolation from human urine the possibility arose that an increase in aldosterone secretion might be responsible for some of these postoperative metabolic events. Several workers who have measured the urinary excretion of aldosterone in patients subjected to major abdominal surgery (not involving the adrenal gland) have found a definite though variable postoperative increase[7,8]. It seemed possible, although unlikely, that a similar postoperative increase might occur after a 2nd adrenalectomy, either as the result of a sudden release at the time of operation or from a subsidiary, non-adrenal source. This would not have been detected by the non-specific methods employed for the determination of urinary steroid in our original observations. We therefore decided to investigate whether or not an increase in aldosterone excretion accompanies the postoperative metabolic response in patients after bilateral adrenalectomy. Our results in three cases are reported in this paper.

METHODS

The balance and chemical methods used were identical with those reported in the previous paper[1]. Aldosterone in urine was determined by the bioassay method of JOHNSON[9] on adrenalectomised rats; the urine was first acidified, hydrolysed, extracted and chromatographed on paper by the procedure of NEHER AND WETTSTEIN[10]. The conversion factor of 36.5 has been used for calculations of aldosterone from the dose response relationship obtained in our adrenalectomised rats using DCA. Our normal values obtained by this method range from 1.1–4.5 μg/24 h; these figures are

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similar to those obtained with a similar technique by VENNING *et al.*[11] (1–6 μg/24 h), LUETSCHER *et al.*[12] (1.8–3.5 μg/24 h), and GENEST *et al.*[13] (0–1.4 μg/24 h). Later workers, using purely physicochemical techniques, have obtained somewhat higher normal values.

The results are shown graphically in Fig. 1 and 2. The data on sodium, potassium, chloride and nitrogen are plotted by the method of REIFENSTEIN, ALBRIGHT AND WELLS[14], in which the intake of each constituent is plotted downwards from the balance line, and urinary excretion is plotted upwards from the intake. Thus, black

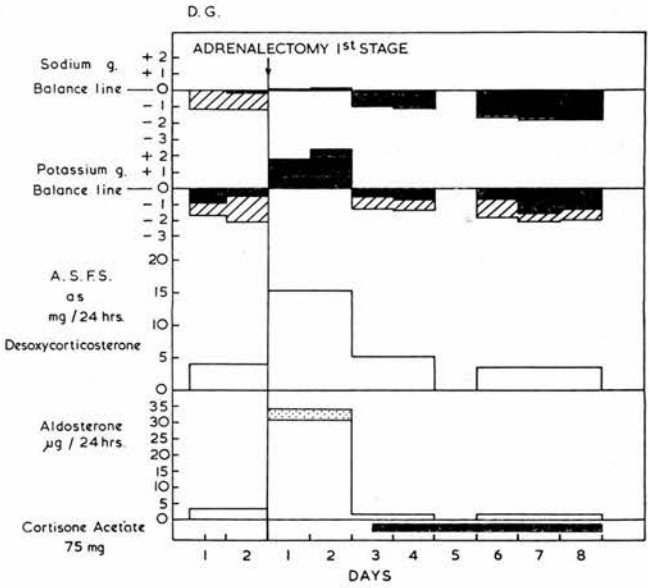

Fig. 1. D.G. First stage adrenalectomy and splanchnicectomy. Black areas above and below the line represent loss and accumulation, respectively.

areas above and below the balance line represent loss and accumulation, respectively. The small and nearly constant losses in the faeces and sweat have been ignored. Urinary total corticosteroids are shown as mg excreted per 24 h (calculated as cortisone), and aldosterone as μg excreted per 24 h.

RESULTS AND DISCUSSION

In the patient D.G. (Fig. 1) observations were made over the first stage of a two-stage adrenalectomy, the patient unfortunately dying a few hours after the second stage. In the immediate postoperative period there was a normal metabolic response with decreased excretion of sodium, increased excretion of potassium, and a four-fold rise in the urinary output of acid-stable formaldehydogenic steroids. The aldosterone excretion was 3.2 μg/24 h pre-operatively and therefore lay within the normal range, and it rose ten-fold on the 1st and 2nd postoperative days. This estimation was confirmed by duplicate analysis with different dosage levels for each rat—the stippled area in the figure representing the difference between the two estimations. On the

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3rd and 4th postoperative days the aldosterone excretion returned to a normal level and remained so on the 6th, 7th and 8th postoperative days, by which time the patient was receiving 75 mg cortisone per day.

The second patient D. McK. (Fig. 2) was followed over the 2nd stage of a bilateral adrenalectomy. During the period of observation beginning seven days before the 2nd stage adrenalectomy, this patient received a constant dose of 200 mg of cortisone

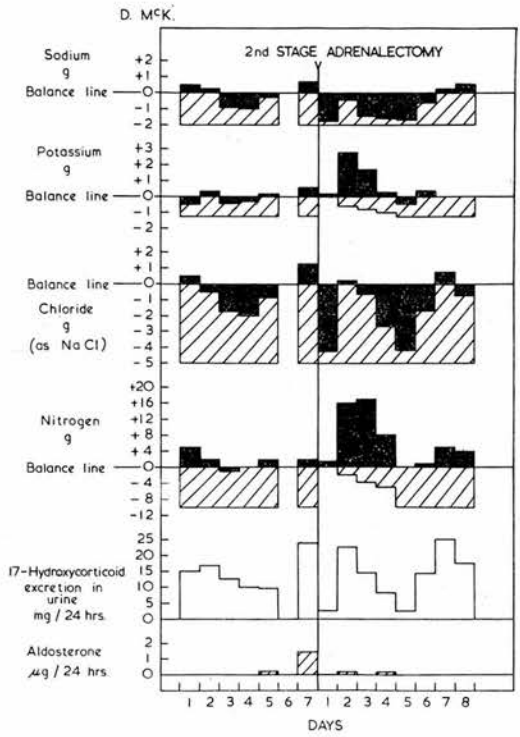

Fig. 2. D.McK. Second stage adrenalectomy and splanchnicectomy. Cortisone acetate 200 mg per day i.m. over period of observation. Black areas above and below the line represent loss and accumulation respectively.

acetate per day in divided doses by intramuscular injection. As in previous instances, the familiar pattern of retention of sodium and chloride, increased excretion of potassium and negative nitrogen balance was obtained, but there was no postoperative rise in the urinary output of either total 17-hydroxy steroids or of acid-stable formaldehydogenic steroids. On the 3rd pre-operative day no aldosterone excretion could be detected, but on the immediate pre-operative day an excretion of 1.6 μg/24 h, *i.e.* an amount within our normal range, was found. No aldosterone whatever could be detected on either the 2nd or 4th post-operative days.

The third patient was maintained on a constant dose of cortisone whilst undergoing an ovariectomy 11 days after the removal of her 2nd adrenal gland. Our data on this case are incomplete as the patient survived for only 2 days after the operation. However, in these 2 postoperative days there was the usual increase in urinary po-

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tassium excretion and reduction in sodium output. No aldosterone could be detected in the urine of this patient on any of the 6 pre- and 2 post-operative days.

There is much evidence to support the view that the biological method for the assay of aldosterone in urine is valid in so far as it is capable of reflecting change in urinary excretion in various physiological and pathological states. However, there exists the possibility that the apparent absence of aldosterone following second stage adrenalectomy in D.McK. might be due to the presence of an inhibitor, though this is, in our view, highly unlikely. GENEST *et al.*[15] found, with different paper chromatography systems, four other substances which have the same R_F values as aldosterone in the Zaffaroni propylene glycol system, and which all absorb U.V. light. In the case of the analyses on D.McK., none of these impurities can have been present in appreciable amounts because no U.V.-absorbing spot was visible.

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Our thanks are due to Professor JOHN BRUCE for his kindness in giving us the opportunity to study patients under his care, and to the Scottish Hospital Endowments Research Trust for a grant which allowed M.S. to take part in these investigations.

SUMMARY

1. Observations have been made on the balance of sodium, potassium, chloride and nitrogen, and the urinary excretion of corticosteroids and aldosterone in three patients undergoing, respectively, 1st stage adrenalectomy, 2nd stage adrenalectomy and ovariectomy after previous bilateral adrenalectomy.

2. Post-operative metabolic changes of sodium and chloride retention with urinary loss of potassium and nitrogen occurred. In the patient subjected to 1st stage adrenalectomy, the metabolic response was accompanied by rises in the urinary excretion of aldosterone and total corticosteroids. In the patient undergoing 2nd-stage adrenalectomy and in the patient undergoing ovariectomy, the metabolic response was not associated with any rise in urinary corticosteroids, and urinary aldosterone was not detectable post-operatively.

3. It is concluded that the metabolic responses ordinarily observed affecting sodium, potassium and nitrogen excretion after surgery can occur without concomitant rises in excretion of aldosterone.

RÉSUMÉ

1. L'équilibre de sodium, de potassium, de chlorure et d'azote, ainsi que l'excrétion urinaire des corticostéroïdes et de l'aldostérone, ont été étudiés dans 3 malades, dont deux d'entre eux étaient soumis au premier, respectivement au deuxième stade d'une surrénalectomie, tandis que la troisième a subi une ovariectomie, précédée d'une surrénalectomie bilatérale.

2. Des changements métaboliques postopératoires du sodium et une rétention de chlorure avec une perte urinaire de potassium et d'azote ont eu lieu. Chez le malade,

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ayant subi le premier stade de surrénalectomie, la réaction métabolique était associée à une augumentation de l'excrétion urinaire de l'aldostérone et de la totalité des corticostéroïdes. Chez le malade, ayant subi le deuxième stade de surrénalectomie, ainsi que chez celle soumise à l'ovariectomie, la réaction métabolique n'était associée à aucune augmentation des corticostéroïdes urinaires, tandis que l'aldostérone urinaire n'était pas décelable après l'opération.

3. Il a été conclu que les réactions métaboliques concernant l'excrétion de sodium, de potassium et de l'azote, observées généralement après une opération, peuvent avoir lieu sans augmentation concomitante de l'excrétion d'aldostérone.

ZUSAMMENFASSUNG

1. Beobachtungen über die Natrium, Kalium, Chlorid und Stickstoffbilanz und die Ausscheidung von Kortikosteroiden und Aldosteron wurden bei drei Kranken, bei welchen entweder das erste Stadium der Adrenalektomie oder das zweite Stadium der Adrenalektomie oder Ovarektomie nach vorausgegangener bilateralen Adrenalektomie durchgeführt wurde, angestellt.

2. Postoperative Stoffwechselstörungen mit Natrium und Chloridzurückhaltung und Verlusten von Kalium und Stickstoff im Urin kamen vor. Bei dem Kranken, der das erste Stadium der Adrenalektomie passiert hatte, war die Stoffwechselreaktion von gesteigerter Ausscheidung von Aldosteron und der totalen Kortikosteroide im Harn begleitet. Bei den zwei anderen Kranken – (zweites Stadium der Adrenalektomie und Ovarektomie) – war die Stoffwechselreaktion mit keiner gesteigerten Ausscheidung der Kortikosteroide im Harn verbunden und Aldosteron konnte im Urin nach der Operation nich nachtgewiesen werden.

3. Es wird der Schluss gezogen, dass die Stoffwechselreaktionen, die gewöhnlich nach chirurgischen Eingriffen beobachtet werden und die Ausscheidung von Natrium, Kalium und Stickstoff betreffen, ohne eine begleitende Steigerung in der Ausscheidungvon Aldosteron stattfinden können.

РЕЗЮМЕ

1. Производилось наблюдение над балансом натрия, калия, хлорида и азота, а также над выбросом в мочу кортикостероидов и альдостерона у трех пациентов из которых один подвергался первой стадии адреналектомии, второй — второй стадии адреналектомии и третий пациент — удалению яичников после предварительной двухсторонней адреналектомии.

2. Имели место послеоперационные метаболические изменения в задержании натрия и хлорида с потерей калия и азота в моче. У больного, подвереннаго первой стадии адреналектомии, метаболическая реакция сопровождалась увеличением выброса альдостерона и кортикостероидов в мочу. У других двух больных метаболическая реакция не была связана с повышением кортикостероидов в моче; альдостерона в моче после операции не наблюдалось.

3. Делается вывод, что обычно наблюдаемая метаболическая реакция, влияющая на послеоперационный выброс натрия и калия, может происходить без повышения выброса альдостерона.

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THE ADRENAL RESPONSE TO SURGICAL TRAUMA.

It has been found by numerous investigators that the complex metabolic changes which follow surgical trauma are associated with an increased urinary excretion of adrenocortical steroids including aldosterone. There has, in consequence, been much discussion as to whether increased adrenocortical secretion is a cause of the metabolic changes in, e.g., electrolyte balance, is permissive of these changes or is merely co-incidental.

Work from this laboratory has shown that the usual metabolic responses (including increased potassium and decreased sodium output) followed removal of the second adrenal gland in patients who, since removal of the first, had been maintained on a constant dose of cortisone; there was, however, no increase in the urinary excretion of corticosteroids.

It has now been found that in these same circumstances the disturbances of electrolyte excretion occurred without increased excretion of aldosterone (as assayed biologically) such as might have occurred, e.g. as the result of an outpouring of pre-formed hormone from the gland at the time of operation.

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